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EFFECTS OF POST-HATCH HOLDING TIME AND EARLY NUTRITION STRATEGIES ON GROWTH PERFORMANCE, CARCASS AND SKELETAL CHARACTERISTICS OF YOUNG CHICKENS

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture at the University of Kentucky

By
Marquisha A. Paul
Lexington, Kentucky

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ABSTRACT OF THESIS

EFFECTS OF POST-HATCH HOLDING TIME AND EARLY NUTRITION STRATEGIES ON GROWTH PERFORMANCE, CARCASS AND SKELETAL CHARACTERISTICS OF YOUNG CHICKENS

The study objectives of this thesis were to evaluate the effects of delayed feeding and specific aspects of the Programmed Nutrition (PN) feeding strategy (Alltech, Inc.) on growth performance, carcass characteristics, and skeletal characteristics of commercial broiler chicks through market age, as well as investigate the effects of breed and the PN feeding strategy on early growth and development. When commercial broiler chicks were fed reduced nutrient diets, delayed feeding decreased early growth performance and carcass yield ($P<0.05$), whereas post-hatch PN conditioning for 72 hours improved early growth performance and alleviated the negative effects of delayed feeding on carcass yield ($P<0.05$). Through market age, delayed feeding improved Gain: Feed ($P<0.05$), while PN had the opposite effect. Interactive effects and main effects of delayed feeding and PN were observed for tissue mineral concentration ($P<0.05$). PN lowered bone ash % ($P<0.05$) and increased meat oxidation of broiler chicks during storage ($P<0.05$). PN also had negative effects on early growth performance and bone breaking strength ($P<0.05$) of various meat-type breeds, but especially for non-commercial, moderate-growing or fast-growing breeds. In conclusion, PN may be suitable for commercial broiler chicks that experience delayed feeding and are fed reduced nutrient diets.

KEYWORDS: Post-hatch delayed feeding, early nutrition strategy, growth performance, carcass characteristics, bone quality

Marquisha A. Paul

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EFFECTS OF POST-HATCH HOLDING TIME AND EARLY NUTRITION STRATEGIES ON GROWTH PERFORMANCE, CARCASS AND SKELETAL CHARACTERISTICS OF YOUNG CHICKENS

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This thesis is dedicated to my son, Malcolm Owen Paul.

You fill my heart with so much love and joy,
by being my funny and special little boy.
You are the reason why I stayed strong,
when the work was hard and the hours were long.
You are the reason I desire to do and achieve more,
the reason behind so many things I strive for,
the reason my passions and dreams are constantly renewed.
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List of Abbreviations

Arg	Arginine
ADBWG	average daily body weight gain
ADFI	average daily feed intake
bursa	Bursa of Fabricius
BWG	body weight gain
°C	degrees Celsius
Ca	Calcium
CO_2	carbon dioxide
CP	crude protein
Cys	Cysteine
d	days
FI	feed intake
g	grams
g/b	grams/bird
g/b/d	grams/bird/day
g/g	grams/grams
Gain:Feed	body weight gain to feed intake ratio
GIT	gastrointestinal
h	hours
HCL	hydrochloric
HNS	hydrated nutritional supplement
HW	hatch window
LNS	liquid nutritional supplement
Lys	Lysine
MDA	Malondialdehyde
ME	metabolizable energy
Met	methionine
n	experimental unit
Na	sodium
NRC	National Research Council

P, avail.	available phosphorus
PN	Programmed Nutrition
PVC	polyvinyl chloride
RH	relative humidity
ROS	reactive oxygen species
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive species
TCA	trichloroacetic acid
Thr	Threonine
Trp	Tryptophan
USDA	United States Department of Agriculture
Vit E	vitamin E
WOG	without giblet
wt.	weight

CHAPTER 1. Literature Review

1.1 Introduction

In commercial broiler operations it is not uncommon for newly hatched chicks to experience delayed access to feed. Delayed access to feed is due to time chicks spend in the hatchery as well as time spent traveling from the hatchery to a grow-out destination. Newly hatched chicks have an internal nutritional reserve, the yolk sac, which can provide chicks with temporary sustenance for up to several days. Despite the presence of the yolk sacs in chicks, delayed feeding can have negative consequences on the growth and development of broiler chicks. Some of these consequences are retarded gastrointestinal and muscular development, reduced growth performance, and immunosuppression (Ao et al., 2012; Decuypere et al., 2001; Dibner et al., 1998; Gonzales et al., 2003; Halevy et al., 2000; Juul-Madsen et al., 2004; Maiorka et al., 2003). The most extreme consequence of delayed feeding is increased mortality.

In response to increased consumer consumption and consumer preference for larger chickens, the broiler industry has targeted specific heritable traits responsible for rapid growth, feed efficiency, and high meat yields during broiler breeding. Therefore, in order for broiler producers to maximize the genetic potential and profitability of these faster growing and larger chickens, they must minimize delayed access to feed as much as possible. Early feeding strategies have been suggested and developed to diminish or possibly reverse the negative effects of delayed feeding. These strategies range from in-ovo feeding to specially designed post-hatch diets (Batal and Parsons, 2002; Leeson, 2008; Uni and Ferket, 2003; Uni and Ferket, 2004).

Energy and nutrient requirements for poultry were last addressed by the National Research Council (NRC) in 1994. The recommendations made by the council are based on research of chicken breeds that were slower growing and less feed efficient than those used in today's broiler industry. Additionally, energy and nutrient requirements for post-hatch growth and development is lacking. Consequently, scientists have worked to redefine modern broiler nutrition requirements. As an added complication, they must address the increased incidences of musculoskeletal and metabolic disorders as well as immunosuppression, unintendedly caused by genetic selection for rapid growth, and which have impacts on production and chicken meat quality.

Animal nutrition and biotechnology companies have developed products supplied as natural or synthetic nutritional feed supplements to improve overall broiler health, production, and meat quality. Some examples of these nutritional feed supplements include antioxidants, enzymes, vitamins, minerals, prebiotics and probiotics. Through the combined use of early feeding strategies and feed technology, it may be possible to overcome the adverse effects of delayed feeding and maximize broiler performance and production potential.

1.2 Post-Hatch Delayed Access to Feed

Newly hatched chicks often experience a delay before receiving access to feed and water because of time spent in the hatchery and time spent traveling to a poultry farm (Careghi et al., 2005). The amount of time chicks spend in the hatchery is largely due to the spread of hatch between early and late hatchers and hatchery processing. On average, it takes approximately 21 days for broiler chicks to completely emerge from their eggs. However, it is estimated that the hatch window (HW), or the span of time from the hatch of the first chicks to the hatch of the last chicks, may range from 24-48 hours or more (Careghi et al., 2005; Decuypere et al., 2001; Noy and Sklan, 1997). In order to minimize loss and for the sake of efficiency, hatchery operators may remove all chicks from the hatchery incubator at one designated time or until most chicks have emerged from their eggs Hager and Beane (1983) and are mostly dried instead of removing the chicks as they hatch. As a result of this common practice, chicks that hatch earlier than others must wait longer periods of time before they receive access to feed and water.

1.2.1 Causes of delayed access to feed: hatch window, hatchery processing, transportation

There is evidence suggesting that breeder flock age, egg characteristics and sex of the embryo may influence the HW. In regard to breeder flock age, progeny from old breeder hens hatch earlier than those from young breeder hens (Ruiz and Lunam, 2002; Ulmer-Franco et al., 2010). When considering egg size, chicks from smaller or lighter eggs may hatch earlier than chicks from larger or heavier eggs (Careghi et al., 2005; Reinhart and Hurnik, 1984; Wilson, 1991). Although hatcheries may strive for uniformity, combining fertile eggs from different breeder flocks and eggs of various sizes may be unavoidable at times and have the consequence of extending the HW. Additionally, sex of the embryo impacts the hatch window. Several studies have indicated that female chicks hatch earlier than male chicks (Burke, 1992; Reis et al., 1997; van de Ven et al., 2011a), yet the effects of delayed feeding appears to be more pronounced in male chicks than female chicks (Hager and Beane, 1983).

The length and temperature of egg storage before incubation can also affect the HW and are variables that hatchery operators can directly control as opposed to breeder flock age, egg size, and sex of the embryo. Chicks hatch earlier from eggs stored for short periods of time compared to eggs stored for a week or more (Mirosh and Becker, 1974; Tona et al., 2003a). Previous work has shown that eggs stored for 1-3 days before incubation can be stored at 18-30°C without affecting the hatch window (Mayes and Takeballi, 1984; Ruiz and Lunam, 2002), whereas storage temperature may have an effect on the HW for eggs stored for longer than 3 days (Ruiz and Lunam, 2002).

During egg incubation, variables such as temperature, humidity and atmospheric gas have an effect on hatch times. In 1937, Barott reported that the best hatch occurs when eggs are incubated at 37.8°C and a few reviews have since confirmed that approximate incubation temperatures of 37-38°C promote optimal hatchability (Bergoug et al., 2013a; Decuypere et al., 2001; Lundy, 1969). Incubation temperatures outside of this range can either prolong or accelerate embryo development and impact the HW (Ande and Wilson, 1981; Yildirim and Yetisir, 2004). Although it has been previously established that 40-70% relative humidity (RH) is an acceptable humidity range during incubation (Barott, 1937; Lundy, 1969), a few studies have demonstrated that a greater percentage of chicks hatched at least 24 hours earlier when incubated at a lower humidity (45% and 50% RH) compared to those incubated at a moderate humidity (57% and 58% RH) or a high humidity (72% and 82% RH) (Reinhart and Hurnik, 1984; Swann and Brake, 1990).

Just as temperature and humidity are regulated in commercial hatchery systems, so is the gaseous environment. Embryos require gas exchange from their environment in order to survive (Barott, 1937). Embryo development under artificial incubation requires the balance and control of the air composition, particularly carbon dioxide (CO_2). Maintaining control of incubator CO_2 levels in the range of 0.1% to 0.4% is commonly practiced in the broiler industry and is normally achieved by ventilation (Bergoug et al., 2013a). Several studies have revealed that embryos exposed to higher levels of CO_2 may hatch earlier (Buys et al., 1998; Everaert et al., 2007) and that higher levels of CO_2 can reduce (P<0.001) the HW by approximately 4.5 hours (De Smit et al., 2006). Additionally, egg handling during the incubation process, specifically egg turning as well as egg position during the latter stage of incubation, can influence the HW by a few hours (Tona et al., 2003b; van de Ven et al., 2011b).

After chicks are removed from hatchers, they may undergo inspection, sorting, sexing, vaccination, and packaging for transport. In commercial hatcheries these processes may add an additional 2 to 4 hours to the time chicks must wait before receiving access to feed and water (Bergoug et al., 2013a). Through the integration and expansion of large hatchery operations, many commercial broiler chicks do not normally travel great distances from the hatchery to a grow-out facility compared to 70 years ago (Bergoug et al., 2013b). According to regulations made by the Council of the European Union in 2005, chicks should be transported to their grow-out destination within 72 hours of hatch, which is similar to recommendations made by broiler breeder companies and experts worldwide. Very little information is available on the average or maximum transportation duration of broiler chicks from hatchery to grow-out facility. A survey of hatcheries conducted in France reported that the average and maximum travel times of broiler chicks are 4 and 10 hours, respectively. One study simulating the road travel conditions and times revealed that 10 hours of travel had a negative effect on chick body weight through 21 days of age when compared to less than 5 minutes or 4 hours of travel. This finding was true only for chicks produced when the breeder flock was younger (35 and 45 weeks old) as opposed to when the flock was older (56 weeks old). By the time broiler chicks reached market age differences in body weights no longer existed (Bergoug et al., 2013b).

Good quality chicks may be defined as chicks with a high performance potential (Tona et al., 2003a; Tona et al., 2005), and in order to maximize that potential, chicks should receive access to feed as soon as possible (Wyatt et al., 1985). In the U.S. commercial broiler industry, nearly 96% of all broilers produced come from large, integrated companies (MacDonald, 2014). This means that they bear the responsibility of maintaining parent breeder flocks, operating hatcheries, as well as shipping chicks from hatcheries to grow out facilities. Therefore, it is in the best interest of these companies to minimize any delay in access to feed so chicks have a better chance at reaching their full performance potential.

1.2.2 Post-hatch yolk sac utilization

Since chicken embryonic growth and development takes place exclusively inside of an egg, the hen must deposit all of the lipids, proteins, vitamins, minerals, and other essential nutrients and substances necessary for chick formation into the egg before it is laid. Hence, the egg yolk serves as one of the main nutritional sources for the developing embryo. As early as the second day of incubation, the yolk sac forms when a thin layer of cells, the blastoderm, begins to encase the egg yolk. The yolk sac quickly becomes a vascularized, extraembryonic membrane which can secrete digestive enzymes for the yolk and facilitate nutrient absorption (Moreng and Avens, 1985). Prior to hatching, the yolk sac is drawn into the abdominal cavity of the embryo (Romanoff, 1960).

According to previous research, the internalized yolk sac accounts for approximately 16% of chick body weight (Chamblee et al., 1992; Heywang and Jull, 1930). Several studies have demonstrated that newly hatched chicks continue to absorb nutrients in the yolk sac, supported by observations of significant decreases in yolk sac weight within the first 3 days of hatch, regardless of being provided immediate access to feed and water (Bierer and Eleazer, 1965; Chamblee et al., 1992; Heywang and Jull, 1930; Mikec et al., 2006). However, at most, the yolk sac contributes to only a portion of the maintenance requirements of the chick during the first several days after hatch (Bigot et al., 2003; Noy et al., 1996) and is incapable of supporting growth.

1.2.3 Consequences of delayed access to feed

Since the 1960's, the U.S. broiler industry has experienced dramatic growth in broiler production and integration, which has been complemented by a large increase of per capita consumption of chicken (MacDonald, 2014). Growth in broiler production has also been concurrent with increases in broiler body weight and growth rate, improvements in the feed conversion rate, and higher meat yields mostly due to genetic selection of such heritable traits (Zuidhof et al., 2014). Based on data collected from the Agricultural Resource Management Survey of the U.S. broiler industry of 2011, within the last decade, consumer demand has also driven a shift towards the production of heavier broilers (USDA et al., 2012).

As of 2011, 97% of commercial broilers produced for meat in the U.S. were raised by contract growers. Many contract broiler growers are provided chicks, feed, and medication from an integrated broiler company. When contract growers have raised the broilers up to market age, they are returned to the broiler company for processing and selling. Contract growers typically receive a predetermined base fee per pound of live-weight produced. They can also make extra earnings or suffer deductions based on contract grower competition and broiler performance. Performance premiums depend on medication costs and mortality, but they depend most heavily on feed conversion, or how efficient broilers convert feed to body weight (MacDonald, 2014). Feed is the largest operating cost in the U.S broiler industry, therefore better feed conversion translates to reduced operation costs and better profit margins for producers. Many studies have demonstrated how delayed access to feed can be detrimental to the development and performance of broiler chicks, which can result in negative consequences for producers.

The first physiological consequence of delayed access to feed is chick body weight loss. In the time between hatch and placement (24-48 hours), chicks may lose an average of 8% of their initial body weight (Casteel et al., 1994; Hager and Beane, 1983; Noy and Sklan, 1999b; Wyatt et al., 1985). Some of the weight loss is due to yolk sac utilization, but it is estimated that up to two thirds of weight loss is due to reductions in tissue and organ weight (Nir and Levanon, 1993). In a recent review by Noy and Uni (2010), it was mentioned that during the hatching process embryos deplete their glycogen reserves which is a nutrient source for the post-hatch chicks. Researchers have hypothesized that there is a shift towards gluconeogenesis, which involves mobilization and metabolism of protein from skeletal muscle for energy. This is thought to be another reason chicks experience weight losses due to post-hatch delayed feeding. Prolonged delayed access to feed (greater than 72 hours) often results in significant increases in chick mortality (El-Husseiny et al., 2008; Misra and Fanguy, 1978).

During the first week after hatch and under normal conditions, chick body weight may increase two to three fold (Bigot et al., 2003), mostly as a result of rapid gastrointestinal (GIT) growth. Gastrointestinal growth occurs at a faster rate compared to other organs and tissues (Noy and Sklan, 1997). Increases in GIT mass are correlated with both the timing of when feed is introduced to chicks and their feed intake (FI) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997) (Noy and Sklan, 1997). When chicks are subjected to delayed feeding of 24-72 hours, GIT growth is stunted and the morphology of the intestinal tract is altered by increasing villus surface area and reducing villus height in the small intestines (Decuypere et al., 2001; Maiorka et al., 2003; Mikec et al., 2006). The altered morphology is thought to be due to unbalanced cell turned over, which arises from excessive cell death and decreased cell renewal (Yamauchi et al., 1996). Results from one trial suggested that gastrointestinal associated lymphoid tissue, especially in the hindgut, may be more susceptible to infectious pathogens during the first two weeks of life when chicks are delayed access to feed (Bar Shira et al., 2005). Consequently, delayed feeding delays GIT development and is thought to lead to less than optimal performance through market age (Gonzales et al., 2003), but it has yet to be proven.

Skeletal muscle growth also contributes to early body weight gain of hatchling chicks. In avian species, skeletal muscle fiber formation is complete at hatch and skeletal muscle growth occurs rapidly thereafter. The first week post-hatch may be the most important time for muscle development, perhaps even more so for broiler strains selected for rapid growth and high meat yield (Halevy et al., 2000; Moss et al., 1964; Simmonds et al., 1964). Specialized myogenic precursor cells found in the skeletal muscle, or satellite cells (Mauro, 1961), play a large role in skeletal muscle growth. Chicks subjected to 48 hours of delayed feeding post-hatch were found to have lagging skeletal muscle fiber development and abnormal satellite cell activity compared to chicks that were fed immediately (Halevy et al., 2000). As a result of delayed feeding body weight and breast muscle weight were significantly depressed at 7 days of age and this observation persisted through market age (41 days). Similar findings have been reported in newly hatched turkey poults subjected to 48 hours of delayed feeding (Halevy et al., 2003).

Long-term negative consequences of delayed feeding on broiler performance through market age have also been documented. Recent investigations have found that only 48 hours of delayed feeding caused reduced body weight through market age (Abed et al., 2011; Bhanja et al., 2009; Gonzales et al., 2003). However, there are conflicting reports as to whether 24 hours of delayed feeding also causes reduced body weight through market age (Casteel et al., 1994; Juul-Madsen et al., 2004; Nir and Levanon, 1993; Vieira and Moran, 1999; Wyatt et al., 1985). For these studies examining the long-term effects of delayed feeding on performance, most reported lower feed intake for chicks that were delayed access to feed, but no differences in the feed conversion rate. This suggests that that despite delayed feeding the chicks were just as efficient in utilizing nutrients, if not more so, than chicks that were allowed immediate post-hatch feeding.

Another long-term consequence of delayed feeding is reduced immune capacity. The bursa of Fabricius (bursa) is an immune organ unique to avian species that produces antibodies in response to pathogen invasion (Glick et al., 1956). Chicks that had been deprived of feed for 48 hours post-hatch had less lymphocyte synthesis after 72 hours, lower bursa weight at 21 days of age, retarded lymphoid development at 21 days of age and lowered disease resistance (Ao et al., 2012; Dibner et al., 1998). Furthermore, 48 hours of post-hatch delayed feeding lowers the immune capacity of broilers up to 42 days of age by way of reduced humoral and cellular immune capacity (Juul-Madsen et al., 2004).

1.3 Early Feeding Strategies

In the U.S. broiler industry, early access to feed and nutrition is widely recognized to have a long-term effect on the development, immune response, and growth performance of broilers. Early development and growth depends on timing of nutrient delivery, nutrient composition of diet, and nutrient density in the diet. Unfortunately, the nutrition requirements for post-hatch chicks have not been clearly defined (Noy and Sklan, 1997; NRC, 1994). This is partially due to different growing objectives of producers. As of 2011, 12% of broilers are marketed as whole chickens, 42% as cut-up parts, and 46% as further processed (National Chicken Council, 2011). Small broilers are marketed as whole chickens or cut-up parts to the fast-food and foodservice sectors. Intermediate sized broilers are marketed as whole chickens or cut-up parts to retail grocery stores. Large broilers are being marketed as roasters or are being further processed into boneless chicken parts, breaded nuggets, chicken sausages, and so forth (MacDonald, 2014).

There is a shift in the U.S. broiler industry towards producing larger, heavier chickens (USDA et al., 2012). In order to maximize weight gain, carcass yields, and feed efficiency, it has been recommended to provide high nutrient dense diets (NRC, 1994; Saleh et al., 2004). Broiler diets are recommended to be more nutrient dense in protein and amino acids, minerals, and vitamins during the starter feeding phase, or up to the first three weeks post-hatch, than any other time. In diet formulation the nutrient composition must also be balanced with energy, since energy is the most costly component of feed. For a long time it has been generally accepted that chickens will consume enough feed to meet their daily energy requirements, provided sufficient nutrients in the diet (Hill and Dansky, 1954). However, it has recently been suggested that optimum growth performance and feed conversion depends on a balanced relationship between dietary nutrient density and energy in feed rations (Saleh et al., 2004).

It has been over 20 years since the nutrient requirements for poultry have been updated (NRC, 1994). Some of the recommendations made by the council were based on research using broiler breeds that were slower growing and less feed efficient than breeds currently used for commercial chicken meat production. Energy and nutrient requirements for post-hatch growth and development is lacking. Since modern broiler breeds have been selected for faster growth, better feed efficiency, and higher meat yields (Havenstein et al., 2003; Schmidt et al., 2009; Zuidhof et al., 2014), more incidences of reduced immunity (Cheema et al., 2003; Yunis et al., 2000), metabolic disorders (Gonzales et al., 1999; Scheele, 1997), and skeletal issues (Rath et al., 2000; Waldenstedt, 2006) have been reported. These are some factors that nutritionists may need to consider when developing early feeding strategies.

In response to the negative effects of delayed feeding on broiler production, early feeding strategies with varying nutritional approaches have been suggested and developed. Some of the early feeding strategies include hatchery feeding or feeding during transport, nutrient injection into the egg during incubation, and feeding pre-starter diets. The nutritional approaches for these early feeding strategies have been based on yolk nutrient composition, embryo nutrient profile, embryo energy and nutrient metabolism, nutrients involved in GIT development, early post-hatch digestibility of simplified and complex diets, or various combinations thereof.

1.3.1 Feeding in the hatchery, during shipment, or both

A common, but perhaps not always practical, early feeding strategy to eliminate or lessen the effects of delayed feeding is to provide feed to chicks while they are still in the hatchery, during transportation, or during both (Careghi et al., 2005). However, because post-hatch nutrient requirements have not been established there are different nutritional approaches that can be taken. This may involve providing a starter diet, a nutritional supplement, or a nutritional supplement with feed additives in hatching trays and/or shipping boxes. Oral gavaing of chicks with a nutrient supplement before chicks are placed at a grow-out facility has also been attempted.

Newly hatched chicks are naturally precocious after they hatch and can learn to eat feed and drink water without assistance. This trait allows chicks to be fed as soon as they hatch. Therefore, studies have been conducted to examine the effects of providing starter diets to broiler chicks and turkey poults while still in the hatchery.

In several experiments, starter diets (NRC, 1994) were placed in the hatching trays of broiler chicks and turkey poults or they were held in the hatching trays without feed for 48 hours (Sklan et al., 2000). As mentioned earlier, not all chicks hatch at the same time due to various reasons. Therefore, investigators evaluated early growth performance through the starter phase based on when chicks hatched during the hatch window. They divided hatchlings into two hatching groups. Early hatchers were considered those that hatched within the first 22 hours of a 48 hour the hatch window, whereas late hatchers were those that hatched during the last 26 hours of the hatch window. The investigators found that at 21 days of age the early hatchers (both broiler chicks and turkey poults) with access to starter diet in their hatcher trays had improved body weight compared to early hatchers that were held without feed for 48 hours. Late hatchers that had been provided starter feed in hatching trays also had improved body weight at 21 days of age, but this was only observed in turkey poults and not broiler chicks. Overall, providing a starter diet in the hatching tray improved body weight as well as uniformity of broiler chicks and turkey poults regardless of sex. Feeding a starter diet immediately after hatch has also been shown to improve body weight through market age (Noy and Sklan, 1999a).

Amino acid nutrition is important for chickens, especially those that have been selected for rapid growth and large breast yield. One of the most important dietary essential amino acids for breast meat accretion in broilers is lysine (Holsheimer and Ruesink, 1993; Kidd et al., 1998; Sibbald and Wolynetz, 1986). It was also found that another essential amino acid for broilers, threonine, interacts with lysine to increase body weight gain and breast meat yield in broilers (Kidd et al., 1997). Total sulfur amino acids (TSAA), which include cysteine and methionine, should also be fed in an appropriate ratio to lysine for best body weight gain and feed conversion (Knowles and Southern, 1998). Chicks also have a high ability to absorb amino acids when they are very young (Batal and Parsons, 2002). A study was conducted to evaluate how hatchery feeding for five hours using starter diets with differing lysine, threonine, and TSAA levels or no hatchery feeding for five hours affected broiler performance (Kidd et al., 2007). Feeding the starter diet for five hours as opposed to no feeding for five hours had no effect on body weight or feed intake of broilers through market age (37 days old), despite any manipulations of amino acid density in the diet. Only marginal improvements in early body weight were observed due to hatchery feeding.

Research has been also conducted feeding early nutritional supplements before placement at a grow-out facility instead of a starter diet. Many post-hatch nutritional supplements available on the market are designed to have a large water component (about 70%) to keep chicks hydrated in addition to providing a source of carbohydrates, protein, fat, and in some instances fiber. Thus, the post-hatch nutritional supplements may be in a semi-liquid form or hydrated. Post-hatch nutritional supplements have been found to be beneficial to broiler performance.

A few studies have been conducted providing a hydrated nutritional supplement (HNS) versus not providing anything to chicks for 48 hours while in hatching trays or shipping boxes. Post-hatch chicks immediately fed an HNS containing 70% water, 20% carbohydrates, 10% protein and <1% fat for 48 hours have been shown to have earlier immune development, better immune resistance, improved growth performance, and enhanced ability to metabolize dietary energy of corn-soybean meal based diets (Batal and Parsons, 2002; Dibner et al., 1998). A similar study using an HNS or liquid nutritional supplement (LNS) product with a slightly different nutrient composition (70% water, 16% carbohydrate, 8% protein, 2% ash, 1% fat, 1% fiber) have demonstrated that immediate administration of a nutritional supplement results in improved early body weight in broiler chicks and turkey poults (Noy and Sklan, 1999a). The improved body weight persisted through market age of broiler chicks. Breast yield was also higher at market age in chicks given the HNS or LNS. Other early feeding experiments have demonstrated that HNS products are able to decrease early body weight losses that chicks normally experienced after hatch and improve muscular satellite cell proliferation, in addition to increasing immune capacity and body weight gain (Henderson et al., 2008).

Chickens may be more susceptible to disease when they are younger because their immune system is still in the process of developing. Disease outbreak on a broiler farm can be costly to producers in terms of treatment costs or loss due to mortality. Therefore, health management is of high importance. Broiler chickens may be vaccinated against certain diseases such as Marek's disease, Newcastle disease, and infectious bronchitis in the hatchery or later on in life. For bacterial diseases for which no vaccination exists, such as Necrotic enteritis, chickens may be treated with antibiotics. Antibiotics are not only administered to broilers to treat diseases, but also to prevent diseases by controlling the pathogenic or opportunistic bacteria that cause them or as a growth promoting feed additive.

Antibiotic use in animals used for food production has become a controversial issue over the years due to growing concerns of microbial resistance to antibiotics. In fact, in early 2006, a ban on use of antibiotics as a growth promotant in animal feed went into effect for countries in the European Union. Some major global fast-food corporations such as McDonald's Corporation have required chicken meat suppliers to discontinue the use of antibiotics as growth promotants, while other global corporations have suffered criticism and increased public scrutiny for not having a public policy on antibiotic use. The pressure on producers to limit antibiotic use has led to an estimated 42% reduction of sub-therapeutic antibiotic use by broiler growers (MacDonald and

Wang, 2011). Consequently, use of prebiotic and probiotic feed additives is becoming more prevalent in the global broiler industry.

Prebiotics are non-digestible source of dietary fiber that promote the growth of beneficial microbiota in the GIT and intended to enhance gut health. Probiotics are living strains of beneficial bacteria that serve the same purpose. A few investigations have been conducted examining the effects of combining an antibiotic, a prebiotic, or probiotic with an HNS on performance and immune resistance against pathogenic bacteria (Ao et al., 2012; Biloni et al., 2013).

Necrotic enteritis is a bacterial disease that affects the small intestine in poultry. It is caused by toxins released by *Clostridium perfringens* (Al-Sheikhly and Truscott, 1977). It is an opportunistic bacterium, meaning that it is commonly found in the GIT and external environment, but becomes harmful under certain conditions. Broiler chicks provided an HNS with a prebiotic immediately after hatch did not exhibit different growth rate or immune resistance after *C. perfringens* challenge in comparison to chicks that were held for 48 hours without access to the HNS with prebiotic (Ao et al., 2012). However, if HNS was combined with an antibiotic (Zinc-bacitracin), chicks were better protected against the challenge.

A separate study combined an HNS (64% water, 22% protein, 20% carbohydrates, 10% fiber, < 2.2% fat) with a probiotic and tested its effectiveness against a *Salmonella* challenge in newly hatched chicks (Biloni et al., 2013). The probiotic consisted of two lactic acid bacterial isolates found in the chicken GIT. Chicks with access to the HNS with probiotic after hatch had improved body weight and GIT morphology as well as less recoverable *Salmonella* in their cecal tonsils compared to chicks that did not have access to the HNS with probiotic.

Alternative to using an HNS that chicks can eat in their hatching trays or shipping boxes, an early nutritional supplement can be administered via crop intubation or oral gavage. The benefits of post-hatch nutrient intubation of chicks on performance have been reviewed by Noy and Uni (2010). Post-hatch crop gavage of starch, which is highly digestible, has been shown to improve chilled carcass yield, breast yield, cellular immune response, bursa weight, and small intestine development in addition to body weight (Bhanja et al., 2010).

1.3.2 In-ovo feeding

In ovo is a Latin term that means in the egg. In 1982, Sharma and Burmester were the first to publish an article describing the successful vaccination of chicken embryos against Marek's disease using *in ovo* injection. Hatchability was unaffected by the new vaccination technique and chicks were protected against the disease post-hatch. A later study revealed that *in ovo* vaccine injection against Marek's disease is most effective when injected directly into the embryo or amniotic fluid (Wakenell et al., 2002). Currently, *in ovo* vaccination is used worldwide in commercial broiler production.

Since the late 1990's, numerous studies have demonstrated that *in ovo* injection of growth factors, antibodies, aromatase inhibitors, feed additives, and nutrients can enhance a variety of characteristics in broilers including skeletomuscular development, GIT development, immune response, growth performance, carcass traits, and meat quality (Bakyaraj et al., 2012; Bhanja and Mandal, 2005; Cheled-Shoval et al., 2011; Coles et al., 1999; Dewil et al., 1998; Hossain et al., 1998; Kadam et al., 2008; Kocamis et al., 1998;

Kocamis et al., 1999; MacDonald and Wang, 2011; Ohta and Kidd, 2001; Ohta et al., 2001; Ohta et al., 1999; Smirnov et al., 2006; Tako et al., 2004; Uni et al., 2005; Uni and Ferket, 2004; Wei et al., 2011; Wu et al., 2000). Most recently, *in ovo* technology has been investigated as an early feeding strategy to deliver nutrients to chicken embryos in an effort to circumvent the negative effects of delayed feeding.

In 2003, Uni and Ferket patented an *in ovo* feeding method (U.S. Patent No. 6592878) to enhance the development of oviparous species that involves injecting a 1 ml liquid nutrient supplement into the embryo amnion several days before hatch. The liquid nutrient supplement is consumed orally by the embryo which stimulates early GIT development. The GIT development of *in ovo* fed chicks at hatch has been shown to be similar to the GIT development of hatchling chicks that had been consuming a conventional starter diet for 2 days and did not receive *in ovo* feeding (Uni and Ferket, 2004). *In ovo* fed chicks also have a better nutritional status which has also been shown to lead to more efficient nutrient utilization and immune capacity. Theoretically, producers may benefit from *in ovo* feeding because production costs can be lowered by using less feed and medication (Uni and Ferket, 2004).

Even though *in ovo* feeding can be beneficial for broiler chickens, *in ovo* nutrition has not been defined. In a recent review of early feeding strategies by Noy and Uni (2010), embryo metabolism during hatch was discussed. They pointed out that during hatching process embryos glycogen reserves are depleted and are not replenished until they are able to feed. Glycogen is the carbohydrate, storage form of glucose and is an easily metabolized source of energy. The yolk sac also serves as a residual energy source, but it cannot support the energy maintenance or growth needs of the chick. As a result, chicks often suffer body weight loss while relying on the yolk sac until they are fed. It is thought that after hatch there is shift towards increasing gluconeogenesis, or metabolism of chick skeletal muscle to produce energy, to compensate for lack of adequate energy supply (Vieira and Moran, 1999). Noy and Uni (2010) also noted that in later stages of embryonic development the brush border enzyme and nutrient transporter activity of the GIT increases. Enhancement of brush border enzymes and nutrient transporter activity also depends on available metabolites such as sodium, chloride, zinc, methionine, and leucine (Smirnov et al., 2006; Tako et al., 2004; Tako et al., 2005).

Based on the information gathered regarding energy and nutrient metabolism leading up to and after hatch, there is some insight as to which nutrients to include when choosing *in ovo* feeding. A recent study demonstrated the long-term impact of *in ovo* feeding and delayed feeding on muscle growth and body weight gain (Kornasio et al., 2011). *In ovo* fed (via amnion) embryos that were delayed feed for 36 hours after hatch had improved market body weight, breast weight, and breast yield compared to chicks that were not *in ovo* fed but also delayed feed for 36 hours after hatch. Glycogen stores in the liver and muscle were also higher due to *in ovo* feeding. When *in ovo* feeding was combined with immediate access to feed, growth and meat yields were further improved (Kornasio et al., 2011). Despite the success of *in ovo* feeding, this method has not gained much popularity (Kadam et al., 2013).

1.3.4 Pre-starter diets

Another response to mitigate the negative effects of delayed feeding and revamp post-hatch nutrition has been to formulate and feed pre-starter diets. A small number of researchers have been successful in showing how pre-starter diets can improve early broiler performance, yet there is wide variation in pre-starter diet formulation. This may be why some studies observed transient effects of pre-starter nutrition on growth performance (Batal and Parsons, 2002; Longo et al., 2007; Swennen et al., 2010), while very few have been able to demonstrate long-lasting improvement on performance through market age (Leeson, 2008; Noy and Uni, 2010).

Leeson (2008) noted that there are two types of pre-starter diets, diets that are either overly high in nutrient content or composed of highly digestible, non-traditional feed ingredients. Dextrose and casein are highly digestible sources of energy and protein, and are utilized especially well during the first week post-hatch when the most dramatic morphological GIT changes occur and when chicks are best able to digest and absorb nutrients (Batal and Parsons, 2002; Sklan, 2001). Leeson also pointed out that high nutrient, pre-starter diets would not be optimally digested and could potentially cause microbial overgrowth. Furthermore, alternative feed ingredients used in pre-starter diets may be highly digestible, but are often more costly than traditional sources of energy and protein, such as corn and soybean.

1.3.5 Programmed Nutrition Feeding Strategy

The Programmed Nutrition (PN) feeding strategy (Alltech, Inc., Nicholasville, KY) is a novel, early feeding strategy that was developed to improve animal performance and production. It employs feeding a PN Post-Hatch Broiler conditioning diet for 72 hours that is designed to induce changes in gene expression associated with energy and nutrient absorption, utilization, metabolism as well as those associated with growth and development. Through conditioning with the PN Post-Hatch Broiler diet, it is hypothesized that chicks gain the ability to adapt to nutrient density changes in their diet later on in life. As part of the PN feeding strategy, chicks are provided PN starter, grower, and pre-harvest diets after the 72 dietary conditioning. The PN starter, grower, and pre-harvest diets are formulated to contain antioxidants, enzymes and organic trace minerals yet are less nutrient dense in calcium, available phosphorus, copper, iron manganese, zinc and Vitamin E, and lower in apparent metabolizable energy than a conventional corn-soybean meal starter, grower and finisher diets (NRC, 1994).

The concept of dietary conditioning can be described as a combination of metabolic programming and imprinting. Metabolic programming has been defined as the metabolic effects of a dynamic process that occurs during a critical time window, whereas as metabolic imprinting are the metabolic changes that occur at the genomic level (Hanley et al., 2010). As previously discussed, early feeding strategies are known to have long-term consequences in broilers. However, it was not revealed until recently that early nutrition has a metabolic imprinting effect on the gene expression of broilers (Brennan et al., 2013).

Broiler chicks that had been fed diets with low levels or normal levels of organic copper, manganese and zinc for 96 hours post-hatch were found to have different intestinal, gene expression profiles when chicks reached 5 days of age (Brennan et al., 2013). From 5 to 21 days of age, all chicks were fed the low, organic mineral diet. Gene expression profiles remained different at 21 days of age due to the long-term effects of the 96 hour post-hatch diets. In addition, chicks fed the low mineral diet for 96 hours post-hatch were significantly heavier than chicks fed the normal mineral diet. Some of the intestinal genes with altered expression included those associated with cellular differentiation, cell proliferation, cellular signaling pathways, and nutrient transport

(Brennan et al., 2013). Although the mechanisms behind these observed changes in gene expression are unknown, proposed mechanisms include transcriptional modifications, clonal selection and metabolic differentiation (Koletzko et al., 2011).

CHAPTER 2 – Effects of delayed feeding and an early feeding strategy with or without dietary conditioning on yolk sac utilization, growth performance, gut morphology, carcass characteristics, bone quality and bone mineral concentration of broiler chicks

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2.1 Abstract

Evidence from emerging studies indicates that an early life nutrition strategy has a long-term impact on growth performance and nutrient metabolism in chicks. It is also well known that delayed access to feed affects post-hatch growth performance in broilers. The Programmed Nutrition (PN) feeding strategy employs feeding a conditioning diet for 72 h after hatch. This strategy is designed to allow chicks to adapt to reduced nutrient diets later on in life. The present study was designed to determine the effects of the delayed feeding and PN feeding strategy with or without dietary conditioning on yolk sac utilization, growth performance, gut morphology, carcass characteristics, bone quality and tibia mineral concentration of broiler chicks. The PN feeding strategy with or without dietary conditioning began on day of hatch, 24 h or 48 h post-hatch. Broiler chicks were randomly assigned to six experimental treatments that were part of a 3x2 factorial design. Stepwise decreases in post-hatch body weight and yolk sac weight were observed as post-hatch holding time increased from 0 to 48 h. Chicks placed on the PN feeding strategy with conditioning were heavier ($P<0.05$) than chicks that were not conditioned at 19 d of age (503 vs 479 g). Post-hatch holding time linearly decreased BWG and FI through d 19. The 48 h holding time decreased FI ($P<0.01$) compared to the other two holding times through 30 d. By 41 d, 48 h holding time decreased FI (*P*<0.05), and tended to improve Gain: Feed (*P*=0.10). Chicks assigned to the PN feeding strategy with conditioning had greater hot (*P*<0.05) and cold (*P*<0.05) carcass yields compared to chicks that did not receive conditioning. Chicks held for 48 h had lower carcass yields (*P*<0.05) and breast tender yields (*P*<0.05). An interactive effect of post-hatch holding time and feeding strategy was observed on the tibia zinc concentration of broilers. There was no effect of post-hatch holding time or feeding strategy on jejunum morphology or bone quality. The results of this experiment indicate that the PN feeding strategy with conditioning improved performance and carcass yield, delayed feeding of 48 hr was detrimental to performance, and delayed feeding along with feeding strategy may influence bone mineral concentration.

Key words: Programmed Nutrition feeding strategy, dietary conditioning, delayed feeding, broiler chicks, growth performance

Introduction

In the commercial broiler industry, broiler chicks often experience a delayed access to feed because of spread of hatch, hatchery processing, and travel from the hatchery to a grow-out facility. The total delay in feed can range from 24 to 48 hours or more (Careghi et al., 2005; Decuypere et al., 2001; Noy and Sklan, 1997). The negative consequences of delayed feed on early body weight loss, growth performance, and early musculoskeletal and gastrointestinal development has been well documented (Abed et al., 2011; Bhanja et al., 2009; Casteel et al., 1994; Decuypere et al., 2001; Gonzales et al., 2003; Mikec et al., 2006; Nir and Levanon, 1993). In response to the negative effects of delayed feeding, early feeding strategies have been developed such as *in ovo* feeding, hatchery feeding, and pre-starter diets, all of which have short-term and long-term effects on growth and development (Batal and Parsons, 2002; Kidd et al., 2007; Noy and Uni, 2010; Sklan et al., 2000; Uni and Ferket, 2004). However, there is very little information available on the effects of delayed feeding or early feeding strategies on carcass characteristics, bone quality, and bone mineral concentration. The purpose of this study was to evaluate the effects delayed feeding on early body weight loss and yolk sac weight loss as well as the effects of delayed feeding and an early feeding strategy with or without dietary conditioning on the growth performance, gut morphology, carcass characteristics, bone quality, and bone mineral concentration of broiler chicks.

2.2 Materials and Methods

The following experiment was conducted in accordance with protocols approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2.1 Animals and Treatments

A total of 1,008 Cobb 500™ male broiler chicks were transported from a local hatchery (Cobb-Vantress, Monticello, KY) in ventilated 24"L x 18"W x 7.5"H cardboard chick shipping boxes (100 chicks/box) with a paper lining and cardboard lid. Upon arrival to the experimental grow-out facility, chicks were randomly assigned to six experimental treatments. The six experimental treatments utilized a 3x2 factorial structure consisting of three post-hatch holding times of 0, 24 and 48 hours and two early feeding strategies. There were seven replicate pens for each experimental treatment with 24 chicks per pen.

It is important to note that there was an estimated 12 hour span from the time when the chicks hatched, were processed at the hatchery, and were transported to the experimental grow-out site. During post-hatch holding (24 and 48 hours only), chicks were kept in the boxes they were transported in and stored in a temperature and light controlled room without access to feed or water. After post-hatch holding, chicks were placed in floor pens with dried pine shavings as bedding along with *ad libitum* access to water and a diet pertaining to their previously assigned early feeding strategy. Water was provided via a nipple drinking system with three nipples per pen. Feed was provided in a hanging tube feeder. The dimensions for each floor pen were 121.92 x 182.88 square centimeters (4 x 6 square feet).

The early feeding strategies were either the PN feeding strategy without conditioning (w/o Conditioning) or the PN feeding strategy with conditioning (w/ Conditioning). The PN feeding strategy without conditioning involved feeding chicks a corn-soybean meal starter diet formulated to meet or exceed chick nutrient and energy requirements (NRC, 1994) for 72 hours (Table 2.1), followed by feeding reduced nutrient

PN Starter, PN Grower, and PN Pre-harvest diets. The PN feeding strategy with conditioning involved feeding chicks a proprietary conditioning diet (PN Post-Hatch Broiler, Alltech, Inc.) for 72 hours followed by feeding the same reduced nutrient PN Starter, PN Grower, and PN Pre-harvest diets (**Figure 2.1**). The PN starter diet was fed through 19 days of age. The PN grower diet was fed from 19-30 days of age. The PN pre-harvest diet was fed from 30-41 days of age.

The PN starter, grower, and pre-harvest diets were formulated to contain antioxidants, enzymes and organic trace minerals (supplemented as Bioplex®) yet have reduced nutrients (0.2% less calcium; 0.2% less available phosphorus; 25% less copper, iron, manganese, and zinc; 20% less vitamin E) and apparent metabolizable energy (75 kcal/kg less) in comparison to a conventional corn-soybean meal diet that meets 1994 NRC nutrient recommendations (**Table 2.2**). The PN starter diet was fed until 19 days of age regardless of post-hatch holding times. Afterwards, the PN grower diet was provided until 30 days of age and then PN pre-harvest diet was provided until 41 days of age.

Separate from the 1,008 chicks that were assigned to conditioning diets, an additional 45 chicks were randomly assigned to only post-hatch holding treatments of 0, 24 or 48 hours specifically for the evaluation of delayed feeding on yolk sac utilization and body weight loss. Each chick was considered a replicate.

2.2.2 Yolk Sac Utilization and Early Body Weight Loss Measurements

Chicks were held for 0, 24 or 48 hours in transport boxes as previously described without access to feed and water. After the designated post-hatch holding time was fulfilled, 15 chicks from each treatment group were removed from their shipping boxes for yolk sac utilization measurements. Individual live body weights were recorded before chicks were euthanized by argon gas asphyxiation and cervical dislocation. Intact yolk sacs were collected and their weights were recorded to calculate yolk sac % (expressed as a percentage of body weight).

2.2.3 Growth Performance Measurements

Chicks were weighed by pen at placement, after the 72 hour dietary conditioning period, and at 19, 30 and 41 days of age in order to measure the average body weight gain (BWG) and average FI (FI). The ratio of BWG to FI (Gain: Feed) was calculated at 19, 30 and 41 days of age.

2.2.4 Gut Morphology Analysis

At 16 days of age, 1 chick per pen was randomly selected and euthanized by argon gas asphyxiation followed by cervical dislocation. The abdominal cavity was opened and 2 cm section of jejunum was removed approximately 2.5 cm proximal to Meckel's diverticulum. Jejunum samples were cut open, rinsed of excess digesta, and fixed in 10% neutral buffered formalin at room temperature for at least 24 hours. The fixed tissue samples were dehydrated in a series of ethanol solutions (70-100% ethanol) and then infiltrated with molten paraffin wax (60°C) using a Thermo Scientific™ STP 120 Spin Tissue Processor. Tissue samples were then embedded into paraffin wax blocks using a Thermo Scientific™ Microm EC 350 Modular Tissue Embedding Center. A rotary microtome (Thermo Scientific™/Microm HM 340E Electronic Microtome) was used to generate 7 µm sections which were adhered to charged, glass microscope slides (1 slide/pen). Tissue slides were stained using a modified Alcian Blue pH 2.5 and Periodic Acid/Schiff's Reagent method (Prophet, 1994). Stained slides were manually coverslipped with DPX mountant (Sigma-Aldrich, St. Louis, Missouri) and photomicrograph images at 40x magnification were captured with a Nikon Eclipse E400 Light Microscope and SPOT Flex Camera. SPOT Basic v5.1 software was used to measure average jejunum villus height and average crypt depth (10 measurements each/slide). Average villus height to crypt depth ratios (VH:CD) were calculated based on villus height and crypt depth measurements.

2.2.5 Bone Quality & Mineral Concentration Analysis

At day 41 of age, 2 birds from each pen were randomly selected and euthanized as previously described. Left tibias were collected and pooled by pen for breaking strength analysis. Bone shafts were cleared of excess soft tissue and breaking strength was measured using an Instron Testing Instrument (Model 4301). Left tibias were placed flat on a raised platform and 100 kg force at a speed of 50 mm/sec was applied with a stainless steel wedge probe positioned perpendicular to center of the bone shafts until they fractured. Right tibias were also collected and pooled by pen for percent ash analysis. Excess soft tissue was removed by boiling in deionized water for 15 minutes. Tibias were then dried at 60°C for a minimum of 12 hours. After drying, tibias were de-fatted in changes of petroleum ether until petroleum ether solution appeared to be free of fat residues. De-fatted tibias were dried overnight at 105°C in a forced air oven and then ashed at 600°C for 6 hours in a muffle furnace. Tibia ash percentage was calculated based on tibia dry weight. Approximately 1 g of tibia ash was microwave digested (CEM Microwave Accelerated Reaction System 5) in 10 ml nitric acid and diluted to 100 ml with deionized water. Diluted samples were analyzed for copper, iron, manganese, zinc, phosphorus, and calcium concentration via Agilent (formerly Varian) Inductively

Coupled Plasma-Optical Emission Spectrum Axial 720 Series (Greenberg and Lynch, 2007).

2.2.6 Carcass Yield Collection

When birds reached 41 days of age, they were transported to a local, USDA approved processing plant (Marksbury Farm, Lancaster, KY) where they were euthanized and processed according to plant procedures. A total of 35 birds per treatment were randomly selected for carcass yield determination. Hot carcass weights without giblets (WOG) were obtain to calculate hot carcass yield (expressed as a percentage of live weight). Hot carcasses were then air chilled to 4.4°C over a period of 16 hours to obtain cold carcass yields (expressed as a percentage of hot carcass weight). The following yields (expressed as a percentage of cold carcass weight) were also obtained: breast filet (pectoralis major - deboned, skinless), breast tender (pectoralis minor), and leg quarter.

2.2.7 Statistical Analysis

Data were subjected to statistical analysis of variance using the general linear model procedures of SAS (v9.3) to determine the main effect of feeding strategy, the main effect of post-hatch holding time and the interactive effects of feeding strategy and post-hatch holding time for all measurements and calculations. Mean values were separated and compared using protected Fisher's least significant difference test. Mean values were declared different for P values < 0.05, with mean values that approached significance ($0.05 \leq P \leq 0.10$) characterized as a tendency to differ.

2.3 Results and Discussion

2.3.1 Yolk Sac Utilization

As post-hatch holding time increased from 0 to 48 hours, a stepwise decrease (P<0.01) in body weight and yolk sac % was observed (**Figure 2.2**). When chicks arrived their average yolk sac constituted 8.4% of chick body weight. Based on previous observations that the yolk sac constitutes up to 16% of the chick body weight (Chamblee et al., 1992; Heywang and Jull, 1930), one can infer that the chicks had used a considerable amount of the yolk sac before the start of the study. Nonetheless, these results agree with published literature regarding yolk sac utilization as well as early body weight loss due to delayed access to feed (Bhanja et al., 2009; Bierer and Eleazer, 1965; Chamblee et al., 1992; Mikec et al., 2006; Nir and Levanon, 1993; Noy and Sklan, 1999b; Noy and Uni, 2010; Vieira and Moran, 1999).

2.3.2 Growth Performance

Main effects of post-hatch holding time

After the 72 hour dietary conditioning period, chicks that were held for 24 or 48 hours had increased (P<0.05) FI and BWG (**Table 2.3**). Previous investigators have shown that 48 hours of delayed feeding is known to induce changes in feeding behavior in chicks, specifically increased appetite (Bigot et al., 2003), which may explain the increased feed consumption. For that particular study, when chicks that were delayed feed, BWG gain was also found to be greater (15 g) in the first 24 hours after receiving feed compared to chicks that received feed immediately. The investigators of that study concluded that the rapid BWG was not solely due to compensatory growth because relative BWG slightly declined after 4 days of age. They reported that large amounts of feed had been stored in the crops of chicks that were delayed feed due to their increased appetite, and attributed this observation to their observed increase in BWG. The results of the present study indicate that initial increased FI and BWG is a direct result of delayed feeding, although it remains unclear if compensatory growth had a role in the initial BWG of chicks that were delayed feed.

By 19 days of age, increasing post-hatch holding times caused a stepwise decrease in both BWG and FI without affecting the Gain: Feed ($P<0.01$) (**Table 2.4**). These findings are in agreement with previous studies (Abed et al., 2011; Bhanja et al., 2009; Gonzales et al., 2003). By 30 days of age, post-hatch holding time no longer had a significant effect on BWG. However, chicks that were held 48 hours had lower FI ($P<0.01$) and higher Gain: Feed ($P<0.01$) (**Table 2.5**). At 41 days of age, chicks held for 48 hours maintained the lowest FI ($P<0.05$) and tended to be more feed efficient ($P=0.10$) (**Table 2.6**). Modern broiler strains are 50% more feed efficient than strains from nearly 60 years ago due to genetic selection (Zuidhof et al., 2014). However, it has been demonstrated that it is possible for male broilers from the same genetic line and fed the same diet to express different feed efficiency phenotypes due to differences in mitochondrial metabolism (Bottje et al., 2006). Furthermore, it has been suggested that delayed feed may alter or mask gene expression (Bigot et al., 2003). Therefore, it is possible that delayed feeding may influence the feed efficiency phenotypes of broilers that are of the same genetic strain.

Main effects of feeding strategy

After the 72 hour dietary conditioning period, chicks that were fed the PN feeding strategy with conditioning had greater BWG ($P<0.05$) and decreased FI ($P<0.05$) intake compared to chicks without conditioning (**Table 2.3**). Through 19 days of age, chicks with conditioning still had greater BWG ($P<0.05$) although FI was not different from chicks that were not conditioned (**Table 2.4**). This is likely due to a carry-over effect of the body weight gains conditioned chicks experienced early on, because by 30 days of age feeding strategy had no significant effect BWG (**Table 2.5**). When chicks reached market age (41 days), there was no effect of feeding strategy on performance (**Table 2.6**). No effects of feeding strategy were observed for Gain: Feed at any time point.

Interactive effects of post-hatch holding time and feeding strategy

There was a tendency for post-hatch holding time and feeding strategy to affect FI at 30 days ($P=0.07$), but by the time chicks were 41 days old there was a significant interactive effect of post-hatch holding time and feeding strategy on FI. As post-hatch holding time increased, a decrease in FI ($P<0.05$) was observed for chicks that were on the PN feeding strategy without conditioning, whereas FI remained the same regardless of post-hatch holding time for chicks that received conditioning (**Table 2.6**). It is possible that PN conditioning promotes greater FI even if post-hatch chicks are delayed access to feed or has some mitigating effect on the reduced FI normally observed due to delayed feeding.

According to the Cobb500 Broiler Performance and Nutrition Supplement (Cobb-Vantress, 2012), the broiler chicks in this study did not reach target body weight or feed efficiency at 41 days of age. For this study, at 41 days of age broilers were performing at the level of 35 day old broiler chickens. In a separate study, chicks fed the PN starter, grower, and pre-harvest diets alone had inferior body weight and feed conversion relative to chicks that received conditioning (Ferket et al., 2013). Furthermore, unbalanced dietary nutrient density and energy may lead to less than optimal growth performance (Saleh et al., 2004). Because there were no differences in BWG or Gain:Feed at the end of the study due to delayed feeding or conditioning, the reduced growth performance may be due to a nutrient deficiency in the PN starter, grower, and pre-harvest diets.

2.3.3 Gut Morphology

Gastrointestinal tract (GIT) development of chicks occurs rapidly within the first week after hatch and contributes greatly to early body weight gain. Several studies have showed that post-hatch GIT development is retarded and the morphology of the small intestines is altered due to delayed feeding (Maiorka et al., 2003; Mikec et al., 2006). Early on, small intestine expansion is rapid and varied, but stabilizes after about 14 days (Noy and Sklan, 1997). Previous studies have only focused on the immediate effects of delayed feeding on GIT development and do not address effects of delayed feeding after growth of the GIT has stabilized (Decuypere et al., 2001; Maiorka et al., 2003; Mikec et al., 2006). At 16 days of age, a tendency towards an interaction between post-hatch holding time and feeding strategy was observed on small intestine morphology (P=, although no main effects of holding time or feeding strategy were observed on jejunum morphology (**Table 2.7**). The first 96 hours of post-hatch nutrition has been found to alter small intestine gene expression in broiler chicks up to 21 days (Brennan et al., 2013). This may explain why there was a tendency of delayed feeding and feeding strategy to have an effect on gut morphology. However, overall, these results may indicate that any physiological changes in gut morphology the chicks would be expected to have early on due to delayed feeding of up to 48 hours disappears around the time GIT development stabilizes.

2.3.4 Carcass Yields

Main effects of post-hatch holding time

There was a decrease in cold carcass yields (*P*<0.05) for chicks that experienced post-hatch holding of 48 hours (**Table 2.8**). The same chicks also had lower breast tender yields (*P*<0.05), but only when compared to chicks that had 24 hour post-hatch holding. These results are in contrast to previous reports that there are no changes in relative carcass yield due to delayed feeding of 24 and 48 hours (Abed et al., 2011; Vieira and Moran, 1999).

Main effects of feeding strategy

Chicks provided the PN feeding strategy with conditioning had significantly greater hot carcass and cold carcass yields (*P*<0.05) compared to chicks that did not receive conditioning, however, no differences in breast filet, breast tender, and leg quarter yields were observed (**Table 2.8**). This could be due to differences in wing and fat yields of the carcasses, which were not measured in this study, or be a direct effect of feeding strategy.

Interactive effects of post-hatch holding and feeding strategy

Interactive effects of post-hatch holding time and feeding strategy were only observed for hot and cold carcass yields (**Table 2.8**). Chicks that experienced 24 hour post-hatch holding with conditioning had greater hot and cold carcass yields (*P*<0.05) compared to chicks that were held for the same amount of time and did not receive conditioning. They also had higher hot and cold carcass yields compared to chicks held for 48 hours, regardless of their conditioning. These results indicate that the PN feeding strategy with conditioning can alleviate the negative effects of 24 hours of delayed feeding on carcass yield.

2.3.5 Bone Quality

There were no effects of delayed feeding and feeding strategy on tibia breaking strength or tibia ash percentage (**Table 2.9**, **Table 2.10**).

2.3.6 Tibia Ash Mineral Concentration

There were no main effects of post-hatch holding time or feeding strategy on tibia ash mineral concentration. An interactive effect of post-hatch holding time and feeding strategy was observed only for tibia ash zinc mineral concentration. For chicks that were held for 0 hours, those with conditioning had higher zinc concentration ($P<0.05$) of tibia ash compared to chicks that were not conditioned (**Table 2.11**). However, chicks that were held for 48 hours and then conditioned exhibited increased zinc concentration in the tibia ash ($P<0.05$) compared to those held for 0 hours and not conditioned. It has been shown in broiler chicks that intestinal zinc transporter gene expression at 21 days of age can be influenced by early feeding strategy (Brennan et al., 2013). It is possible that the timing of a post-hatch early feeding strategy has a role in metabolic imprinting and can alter how zinc is absorbed later on in life.

2.4 Conclusions

Delayed feeding causes post-hatch body weight loss, reduced early body weight gain and feed intake, as well as reduced carcass yield of market age birds. The Programmed Nutrition feeding strategy with conditioning improved early body weight gain and alleviated the negative effects of post-hatch holding time on carcass yield. However, the lower than recommend levels of metabolizable energy and nutrient concentration of the PN starter, grower, and pre-harvest diets may have prevented chicks from reaching their full growth performance potential. Even though there were no effects of delayed feeding and feeding strategy on bone quality, there was an interactive effect between delayed feeding and early feeding strategy on bone zinc absorption.

2.5 Figures and Tables

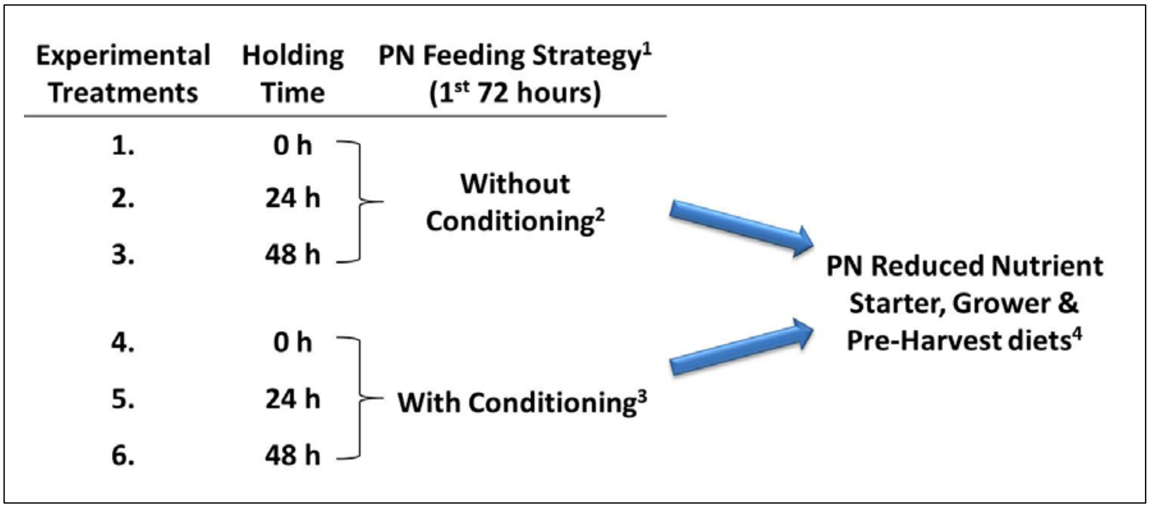


Figure 2.1 Experimental treatment structure for post-hatch holding time and PN feeding strategy with or without 72 hour dietary conditioning

[1]Upon the completion of designated holding time, chicks were assigned to a PN feeding strategy without or with conditioning

[2]Chicks were provided a corn-soybean meal based starter diet that met or exceeded poultry nutrient requirements (NRC, 1994) for 72 hours

[3]Chicks were provided a proprietary conditioning diet (PN Post-Hatch Broiler, Alltech, Inc.) for 72 hours

[4]Provided after finishing the 72 hour conditioning period

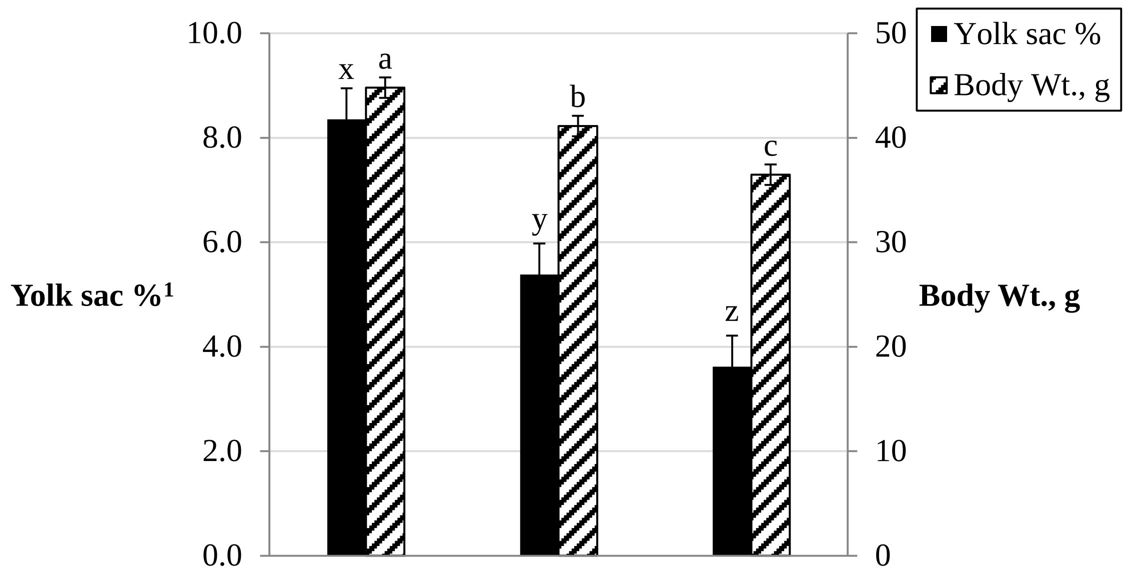
Table 2.1 Composition and calculated analysis of starter diet fed for 72 hours to chicks assigned to the PN feeding strategy without conditioning

Diet Composition	%
Corn	57.00
Soybean meal (48% CP)	36.00
Vegetable oil	2.82
Limestone	1.42
Dicalcium Phosphate	1.75
Salt	0.45
L-Lysine HCL	0.10
DL-Methionine	0.21
Vitamin-mineral premix	0.25
Total	100.00
Calculated Analysis	
ME, kcal/kg	3036
CP, %	22.52
Ca, %	1.03
P, avail., %	0.46
Na, %	0.19
Arg, %	1.54
Lys, %	1.29
Met, %	0.55
Met+Cys, %	0.91
Thr, %	0.84
Trp, %	0.30

Table 2.2 Composition and calculated analysis of PN starter, grower, and pre-harvest diets

Diet Composition, %	PN Starter[1]	PN Grower[2]	PN Pre-harvest[3]
Corn	59.93	65.44	68.28
Soybean meal (48% CP)	35.50	30.40	26.10
Vegetable oil	1.00	0.86	1.60
Limestone	1.47	1.37	1.37
Dicalcium Phosphate	0.67	0.50	0.40
Salt	0.46	0.45	0.45
L-Lysine HCL	0.11	0.09	0.00
DL-Methionine	0.21	0.24	0.15
Vitamin premix (no Vit E)	0.25	0.25	0.25
PN Premix	0.40	0.40	0.40
Celite	0.00	0.00	1.00
Total	100.00	100.00	100.00
Calculated Analysis			
ME, kcal/kg	2961	3017	3059
CP, %	22.52	20.52	18.58
Ca, %	0.83	0.73	0.70
P, avail., %	0.26	0.22	0.19
Na, %	0.19	0.19	0.19
Arg, %	1.54	1.39	1.26
Lys, %	1.29	1.15	0.96
Met, %	0.55	0.56	0.44
Met+Cys, %	0.91	0.89	0.75
Thr, %	0.84	0.76	0.69
Trp, %	0.30	0.27	0.24

[1]Fed following the 72 hour dietary conditioning period and through 19 days of age.
[2] Fed from 19-30 days of age.
[3]Fed from 30-41 days of age.



	0 h	24 h	48 h	SEM
Yolk Sac, %[1]	8.36	5.38	3.62	0.59
Body Wt., g	44.8	41.1	36.5	0.97

Figure 2.2 Effect of post-hatch holding time on chick yolk sac % and body wt. (n=15)
[1]Expressed as a percentage of body wt.
[a-c]Yolk sac % means without a common letter are different (*P*<0.01).
[x-z]Body Wt. means without a common letter are different (*P*<0.01).

Table 2.3 Effects of post-hatch holding time and feeding strategy on body weight gain and feed intake of broiler chicks after the 72 hour conditioning period*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b
w/o Conditioning	33.1^{b}	39.0^{a}
w/ Conditioning	40.4^{a}	33.2^{b}
SEM	0.53	0.40
P-value	<0.01	<0.01
Holding Main Effects		
0 h	33.7^{c}	34.5^{b}
24 h	37.2^{b}	37.1^{a}
48 h	39.3^{a}	36.8^{a}
SEM	0.65	0.49
P-value	<0.01	<0.01
Interactive Effects		
0h holding w/o Conditioning	29.9^{z}	37.6
24h holding w/o Conditioning	32.6^{y}	40.3
48h holding w/o Conditioning	36.8^{x}	39.1
0h holding w/ Conditioning	37.6^{x}	31.4
24h holding w/ Conditioning	41.9^{w}	33.8
48h holding w/ Conditioning	41.7^{w}	34.5
SEM	0.92	0.70
P-value	0.06	0.34

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

abcMeans within the same column without common letters are different (P<0.05).

wxyzMeans within the same column without common letters tend to differ (0.05≤P≤0.10).

Table 2.4 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-19 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain: Feed, g/g
w/o Conditioning	479[b]	756	0.65
w/ Conditioning	503[a]	745	0.68
SEM	6.68	15.3	0.02
P-value	0.02	0.61	0.21
Holding Main Effects			
0 h	528[a]	824[a]	0.65
24 h	485[b]	764[b]	0.64
48 h	461[c]	662[c]	0.70
SEM	8.19	18.7	0.02
P-value	<0.01	<0.01	0.12
Interactive Effects			
0h holding w/o Conditioning	515	841	0.63
24h holding w/o Conditioning	466	780	0.60
48h holding w/o Conditioning	457	647	0.71
0h holding w/ Conditioning	540	808	0.67
24h holding w/ Conditioning	504	749	0.67
48h holding w/ Conditioning	465	678	0.69
SEM	11.6	26.5	0.03
P-value	0.45	0.40	0.29

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

[abc]Means within the same column without common letters are different ($P<0.05$).

Table 2.5 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-30 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain: Feed, g/g
w/o Conditioning	1162[y]	1978	0.593
w/ Conditioning	1191[x]	1952	0.611
SEM	11.57	28.08	0.009
P-value	0.087	0.522	0.148
Holding Main Effects			
0 h	1197[x]	2070[a]	0.583[b]
24 h	1179[xy]	1998[a]	0.592[b]
48 h	1153[y]	1827[b]	0.631[a]
SEM	14.17	34.39	0.011
P-value	0.095	<0.001	0.008
Interactive Effects			
0h holding w/o Conditioning	1199	2145[x]	0.564
24h holding w/o Conditioning	1146	1997[y]	0.576
48h holding w/o Conditioning	1142	1790[z]	0.638
0h holding w/ Conditioning	1196	1994[y]	0.602
24h holding w/ Conditioning	1213	1998[y]	0.608
48h holding w/ Conditioning	1164	1864[yz]	0.625
SEM	20.04	48.63	0.016
P-value	0.232	0.074	0.216

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

[abc]Means within the same column without common letters are different ($P<0.05$).

[xyz]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

Table 2.6 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-41 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain:Feed, g/g
w/o Conditioning	1888	3444	0.550
w/ Conditioning	1923	3420	0.559
SEM	15.51	39.07	0.005
P-value	0.121	0.661	0.269
Holding Main Effects			
0 h	1903	3484[a]	0.543[y]
24 h	1918	3496[a]	0.549[y]
48 h	1896	3316[b]	0.572[x]
SEM	18.99	47.85	0.007
P-value	0.711	0.019	0.010
Interactive Effects			
0h holding w/o Conditioning	1916	3615[a]	0.532
24h holding w/o Conditioning	1874	3466[ab]	0.542
48h holding w/o Conditioning	1874	3253[c]	0.576
0h holding w/ Conditioning	1891	3353[bc]	0.553
24h holding w/ Conditioning	1961	3527[ab]	0.556
48h holding w/ Conditioning	1917	3380[bc]	0.567
SEM	26.86	67.67	0.009
P-value	0.125	0.015	0.263

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

[abc]Means within the same column without common letters are different (P<0.05).

[xy]Means within the same column without common letters tend to differ (0.05≤P≤0.10).

Table 2.7 Effects of post-hatch holding time and feeding strategy on jejunum morphology of broiler chicks at 16 days of age*

Feeding Strategy Main Effects	Villus Height, µm	Crypt Depth, µm	VH:CD[1]
w/o Conditioning	825	175	4.96
w/ Conditioning	786	173	4.92
SEM	17.4	5.11	0.18
P-value	0.12	0.80	0.89
Holding Main Effects			
0 h	823	180	4.81
24 h	780	173	4.97
48 h	813	169	5.05
SEM	21.7	6.26	0.22
P-value	0.38	0.46	0.74
Interactive Effects			
0h holding w/o Conditioning	831[xy]	186	4.71
24h holding w/o Conditioning	774[y]	170	4.73
48h holding w/o Conditioning	871[x]	169	5.44
0h holding w/ Conditioning	815[xy]	174	4.91
24h holding w/ Conditioning	787[xy]	177	5.21
48h holding w/ Conditioning	755[y]	169	4.65
SEM	30.1	8.86	0.31
P-value	0.10	0.56	0.12

*Mean morphology values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7); each pen average represents 10 measurements for villus height, crypt depth, and VH:CD.

[xy]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

[1]VH:CD = villus height to crypt depth ratio.

Table 2.8 Effects of post-hatch holding time and feeding strategy on carcass yields of broiler chicks at 41 days of age[*]

Feeding Strategy Main Effects	Hot Carcass[1]	Cold Carcass[1]	Breast filet[2]	Breast tender[2]	Leg Quarter[2]
w/o Conditioning	73.0[b]	71.3[b]	22.6	4.25	28.7
w/ Conditioning	73.5[a]	71.6[a]	22.3	4.24	28.5
SEM	0.03	0.02	0.02	0.00	0.02
P-value	0.02	0.044	0.33	0.90	0.11
Holding Main Effects					
0 h	73.4	71.6[a]	22.5	4.22[ab]	28.7
24 h	73.4	71.6[a]	22.4	4.34[ab]	28.5
48 h	73.1	71.1[b]	22.5	4.17[b]	28.6
SEM	0.02	0.03	0.00	0.01	0.02
P-value	0.56	<0.05	1.00	<0.05	0.39
Interactive Effects					
0h holding w/o Conditioning	73.3[ab]	71.5[b]	22.6	4.23	28.8
24h holding w/o Conditioning	72.8[b]	71.1[b]	22.4	4.31	28.6
48h holding w/o Conditioning	73.1[b]	71.2[b]	22.7	4.20	28.8
0h holding w/ Conditioning	73.4[ab]	71.6[ab]	22.3	4.20	28.7
24h holding w/ Conditioning	74.0[a]	72.1[a]	22.5	4.38	28.3
48h holding w/ Conditioning	73.2[b]	71.1[b]	22.2	4.14	28.4
SEM	0.41	0.41	0.17	0.09	0.19
P-value	0.04	0.03	0.68	0.60	0.78

[*]Mean values represent pen averages (Feeding Strategy main effects, n=105; Holding main effects, n=70; Interactive effects, n=35).
[1]Values expressed as a percentage of live weight.
[2]Values expressed as a percentage of cold carcass weight.
[ab]Means within the same column without common letters are different ($P<0.05$).

Table 2.9 Effects of post-hatch holding time and feeding strategy on tibia breaking strength of broiler chicks at 41 days of age*

Feeding Strategy Main Effects	Tibia breaking strength, kg force
w/o Conditioning	32.34
w/ Conditioning	32.71
SEM	1.017
P-value	0.795
Holding Main Effects	
0 h	32.61
24 h	34.37
48 h	30.60
SEM	1.246
P-value	0.119
Interactive Effects	
0h holding w/o Conditioning	33.51
24h holding w/o Conditioning	31.71
48h holding w/o Conditioning	33.79
0h holding w/ Conditioning	34.94
24h holding w/ Conditioning	29.70
48h holding w/ Conditioning	31.42
SEM	1.762
P-value	0.559

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

Table 2.10 Effects of post-hatch holding time and feeding strategy on tibia ash percentage of broiler chicks at 41 days of age*

Feeding Strategy Main Effects	Tibia ash, %
w/o Conditioning	47.76
w/ Conditioning	47.55
SEM	0.543
P-value	0.789
Holding Main Effects	
0 h	48.37
24 h	47.38
48 h	47.23
SEM	0.665
P-value	0.431
Interactive Effects	
0h holding w/o Conditioning	48.33
24h holding w/o Conditioning	48.40
48h holding w/o Conditioning	47.76
0h holding w/ Conditioning	46.99
24h holding w/ Conditioning	47.19
48h holding w/ Conditioning	47.26
SEM	0.941
P-value	0.876

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

Table 2.11 Effects of post-hatch holding time and feeding strategy on mineral concentration of broiler chick tibia ash at 41 days of age *

Feeding Strategy Main Effects	Cu, mg/kg	Mn, mg/kg	Fe, mg/kg	Zn, mg/kg	Ca, %	P, %
w/o Conditioning	3.58	5.60	429^{y}	339	35.7	17.8
w/ Conditioning	3.63	5.79	454^{x}	340	35.6	17.8
SEM	0.07	0.26	10.3	4.06	0.06	0.05
P-value	0.66	0.61	0.10	0.77	0.21	0.38
Holding Main Effects						
0 h	3.56	6.04	449	337	35.8	17.8
24 h	3.56	5.63	429	334	35.6	17.8
48 h	3.68	5.42	447	348	35.7	17.8
SEM	0.09	0.31	12.7	4.97	0.07	0.06
P-value	0.57	0.34	0.50	0.14	0.40	0.91
Interactive Effects						
0h holding w/o Conditioning	3.45	5.66	437	325^{c}	35.7	17.7
24h holding w/o Conditioning	3.49	5.74	398	332^{bc}	35.8	17.9
48h holding w/o Conditioning	3.81	5.41	451	359^{a}	35.7	17.9
0h holding w/ Conditioning	3.68	6.43	460	348^{ab}	35.6	17.9
24h holding w/ Conditioning	3.64	5.52	460	336^{bc}	35.8	17.7
48h holding w/ Conditioning	3.55	5.42	442	337^{bc}	35.6	17.8
SEM	0.13	0.44	17.9	7.01	0.10	0.08
P-value	0.12	0.51	0.17	0.01	0.30	0.23

*Mean values represent pen averages (Feeding Strategy main effects, n=21; Holding main effects, n=14; Interactive effects, n=7).

abcMeans within the same column without common letters are different ($P<0.05$).

xyMeans within the same column without common letters tend to be different ($0.05 \leq P \leq 0.10$).

CHAPTER 3 – Effects of delayed feeding and Programmed Nutrition feeding strategy on growth performance, tissue mineral concentration, carcass yields and meat quality of broiler chicks

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3.1 Abstract

Previous research has indicated that nutritional experiences in early life may have a long-lasting effect on nutrient absorption and growth performance in chicks. The Programmed Nutrition (PN) feeding strategy uses a post-hatch diet to condition chicks for optimal nutrient absorption and to adapt to ration nutrient density changes throughout life. The purpose of this study was to investigate the effects of delayed feeding and PN feeding strategy on the growth performance, tissue mineral concentration, carcass yields and meat quality of broiler chicks. A 3x2 factorial treatment arrangement was utilized to assess the effects of post-hatch delayed feeding (0 h, 24 h, or 48 h) and the PN feeding strategy versus a control feeding strategy on growth performance. Early BWG was increased (P<0.05) for chicks fed the PN feeding strategy and decreased (P<0.05) for chicks that had 24 or 48 hours of delayed feeding. By the time the broiler chicks reached market age, PN fed birds had decreased Gain: Feed (P<0.05), whereas chicks that had 48 hours of delayed feed had improved Gain: Feed (P<0.05). The PN fed chicks had higher wing yield (P<0.05), lower tibia ash % (P<0.05), and higher meat oxidation over time (P<0.05). Delayed feeding and PN feeding strategy altered bone ash and breast tissue mineral concentration. The results of this study demonstrated that the PN feeding strategy and delayed feeding are able to alter early growth performance and have long-term effects on tissue mineral concentration, carcass yields, and tissue mineral concentration.

Key words: Programmed Nutrition Feeding Strategy, delayed feeding, broiler chicks, performance, meat quality, tissue mineral concentration

Introduction

It is common in the commercial broiler industry for newly hatched chicks to experience delayed access to feed due to of spread of hatch, hatchery processing, and travel from the hatchery to a grow-out facility. It is estimated that chicks must wait 24 to 48 hours or more until they first receive access to feed (Careghi et al., 2005; Decuypere et al., 2001; Noy and Sklan, 1997). The negative effects of delayed feed on early body weight loss, growth performance, and early musculoskeletal and gastrointestinal development have been well documented (Abed et al., 2011; Bhanja et al., 2009; Casteel et al., 1994; Decuypere et al., 2001; Gonzales et al., 2003; Mikec et al., 2006; Nir and Levanon, 1993). In response to the negative effects of delayed feeding, early feeding strategies have been developed such as *in ovo* feeding, hatchery feeding, and pre-starter diets, all of which have short-term and long-term effects on growth and development (Batal and Parsons, 2002; Kidd et al., 2007; Noy and Uni, 2010; Sklan et al., 2000; Uni and Ferket, 2004). However, there is very little information available on the effects of delayed feeding or early feeding strategies on carcass characteristics, bone quality, tissue mineral status, and meat quality. The purpose of this study was to evaluate the effects delayed feeding and an early feeding strategy on the growth performance, gut morphology, carcass characteristics, bone quality, tissue mineral status, and meat quality of broiler chicks.

3.2 Materials and Methods

The following experiment was conducted in compliance with the protocols set forth and approved by the University of Kentucky Institutional Animal Care and Use Committee.

3.2.1 Animals and Treatments

A total of 1,152 Cobb 500™ male broiler chicks were transported from a local hatchery (Cobb-Vantress, Monticello, KY) in ventilated 24”L x 18”W x 7.5”H cardboard chick shipping boxes of 100 chicks per box. Each shipping box was paper lined and had a cardboard lid. There is an estimated 12 hour span from the time chicks hatched, were processed, and transported to the experimental grow-out site. Once chicks arrived to the experimental grow-out facility, they were randomly assigned to 6 experimental treatments. The 6 experimental treatments utilized a 3x2 factorial arrangement which consisted of 3 post-hatch holding times and 2 feeding strategies. There were 8 replicate pens for each experimental treatment with 24 chicks per pen. During post-hatch holding, chicks were kept in their shipping boxes and stored in a room that was light and temperature controlled for 0, 24 or 48 hours without access to feed or water. After post-hatch holding, chicks were transferred to floor pens with dried pine shavings as bedding. The dimensions for each floor pen were 121.92 x 182.88 square centimeters (4 x 6 square feet). In the floor pens, chicks were assigned to either a control feeding strategy (control) or Programmed Nutrition (Alltech, Inc., Nicholasville, KY) feeding strategy (PN). Both feeding strategies included a 72 hour dietary conditioning period where chicks were provided *ad libitum* access to feed and water. Water was provided via a nipple drinking system with three nipples per pen and feed was provided in a hanging tube feeder.

Chicks in the control group were fed the control starter diet for the 72 hour conditioning period, whereas chicks in the PN group were fed PN Post-Hatch Broiler diet.

After 72 hours of dietary conditioning, chicks were fed starter, grower and finisher diets that corresponded to their assigned feeding strategy. The control group starter, grower and finisher diets were formulated to meet nutrient and energy requirements as set forth by theNRC (1994). The PN group starter, grower, and finisher diets were formulated to contain antioxidants, enzymes, and organic trace minerals, and a lower density of nutrients (0.1% less calcium; 0.1% less available phosphorus; 25% less copper, iron, manganese, and zinc; 20% less vitamin E) and less energy content (75 kcal/kg less AME_n), compared to the control diets (**Table 3.1**).

Due to post-hatch holding times, all chicks were allotted the same amount of time on the starter and grower diets which were 15 and 17 days, respectively. Since the feeding portion of the trial ended after 42 days, the finisher diets were only provided for 7, 6, and 5 days for respective post-hatch times of 0, 24, and 48 hours.

3.2.2 Growth Performance Measurements

Chick body weight (by pen) was recorded at placement. After the 72 hour dietary conditioning period, the average body weight gain (BWG) and feed intake (FI) were measured. At 19, 36 and 42 days of age BWG and FI were measured and used to calculate the average body weight gain to feed intake ratio (Gain: Feed). Due to post-hatch holding time, growth performance measurements were also obtained after each feeding phase to assess growth performance based on time on feed. All chicks were allowed 15 days of access to the starter diets and 17 days of access to the grower diets. Since the feeding trial feeding was ended on 42 days of age, chicks were not allowed the same amount of access to the finisher diet. Chicks that had been held for 0, 24 or 48 hours were allotted 7, 6, and 5 days on the finisher diets, respectively. Growth performance measurements by the starter, grower and finisher feeding phases were calculated as average daily body weight gain (ADBWG), average daily feed intake (ADFI), and Gain: Feed.

3.2.3 Carcass Characteristics and Meat Sample Collection

When chicks reached 43 or 44 days of age, one chick per pen was euthanized via electrical stunning followed by exsanguination in accordance with University of Kentucky IACUC approved procedures. Euthanized chicks were briefly immersed in a hot water and then de-feathered using a semi-automated chicken plucker. The digestive tract, giblets (heart, liver, gizzard, and neck), lungs, feet, and shanks were removed. Hot carcass without giblets (WOG) and abdominal fat (fat pad) weights were collected to calculate their respective yields (expressed as a percentage of live weight). Hot carcasses were then chilled in ice water for approximately three hours to obtain cold carcass yields (expressed as a percentage of hot carcass weight). Breast filet (pectoralis major - deboned, skinless), breast tender (pectoralis minor), wing and leg quarter weights were collected from each cold carcass to calculate yields. Breast filets were retained and stored on ice until meat quality analysis was performed. Individual chicken thighs were also retained and packaged into sealed plastic freezer bags that were stored at -10°C for five months for meat quality analysis.

3.2.4 Tissue Sample Collection and Analysis

At 42 days of age, 2 birds from each pen were randomly selected and euthanized by argon gas asphyxiation followed by cervical dislocation. Approximately 5 g of pectoralis major tissue was collected, homogenized and stored at -20°C until mineral analysis could be performed. Left tibias and humeri were collected and pooled by pen for breaking strength analysis via Instron Testing Instrument (Model 4301). Excess soft tissue was removed from the bone shaft prior to analysis of breaking strength. Bones were placed flat on a raised platform where a stainless wedge probe aligned perpendicular to the center of the bone shafts applied 100 kg force at a speed of 50mm/sec until the bones fractured. Right tibias and humeri were also collected and pooled by pen for percent ash analysis. Bones were boiled in deionized water for 15 minutes to remove flesh and dried at 60°C for a minimum of 12 hours. Bones were then de-fatted in changes of petroleum ether until petroleum ether solution appeared to be free of fat residues. De-fatted bones were dried overnight at 105°C in a forced air oven and then ashed at 600°C for 6 hours in a muffler furnace. Ash percent was calculated on a dry matter basis.

3.2.5 Tissue Mineral Concentration Analysis

Approximately 1 g of tibia ash or 1 g of pectoralis major (wet basis) was microwave digested (CEM Microwave Accelerated Reaction System 5) in 10 ml nitric acid and diluted to 100 ml with deionized water. Digested tissue samples were analyzed for copper, iron, manganese, zinc, phosphorus, and calcium concentration with Agilent (formerly Varian) Inductively Coupled Plasma-Optical Emission Spectrum Axial 720 Series (Greenberg and Lynch, 2007).

3.2.6 Meat Quality Analysis

Previously collected left breast filets (1 breast filet/pen) were weighed and placed in sealed, gallon sized, plastic storage bags. Bagged breast filets were suspended and stored at 4°C with constant exposure to 200 watts of incandescent light. Breast filets were weighed on day 3 and day 7 for drip loss determination (expressed as percentage of original filet weight). Right breast filets (1breast filet/pen) were used to measure the oxidative stability of raw meat and chicken thighs (1 chicken thigh/pen) were used to measure the oxidative stability of frozen, stored meat according to similar procedures used by Quant (2012). Previously frozen chicken thighs were thawed for 24 hours at 4°C and deboned before analysis. Both breast filets and thighs were cut into three equal sections. One section was used immediately for thiobarbituric acid reactive species (TBARS) assay to determine oxidative stability, while each remaining section was separately placed onto 8” x 5-3/4” x 3/4” white foam trays containing a 3-1/3” x 6” reversible soaker pad and wrapped with polyvinyl chloride (PVC) film. Packaged meat sections were stored in a retail cooler set to 2°C under 1300 lux fluorescent lighting until TBARS assay was performed at 3 and 7 days of storage. For the TBARS assay, approximately 5 g of meat was homogenized in 22.5 ml of 11% trichloroacetic acid (TCA) solution using an Ultra-Turrax® T25 rotor-stator homogenizer and saw tooth dispersing element (IKA® Works, Inc., Wilmington, NC). The homogenate was filtered through Whatman #1 filter paper and 1 ml of homogenate was mixed with 1 ml of 20 mM thiobarbituric acid (TBA). A blank was prepared by mixing 2ml of 11% TCA solution with 2ml of 20 mM TBA solution. The resulting sample and blank solutions were incubated at 25°C for 20 hours. After incubation, the absorbance of the malondialdehyde (MDA) in the solution was read at 532 nm on a Beckman-Coulter DU 730 Life Science UV/Visual Spectrophotometer. Given the MDA extinction coefficient factor of 1.56 x 10^5 $M^{-1}cm^{-1}$, the concentration of MDA (expressed as mg MDA/kg meat) was calculated based upon the Beer's Law (1852) and the following equation:

$$MDA\ Concentration = \frac{ABS}{Length\ (1) x\ K} \times \frac{1L}{Tissue\ Concentration}$$

Where, ABS refers to absorbance of MDA at 532nm, Length refers to path length of the sample, K (a constant) refers to the product of the extinction coefficient factor and molecular weight of MDA, and Tissue Concentration accounts for the concentration of the meat sample after homogenization and dilution in TCA and TBA solutions.

3.2.7 Statistical Analysis

Analysis of variance for a 3x2 factorial arrangement of treatments was conducted using the general linear model procedures of SAS (v9.3). This analysis allowed for the determination of the main effect of feeding strategy, the main effect of post-hatch holding time and the interactive effects of feeding strategy and post-hatch holding time for all measurements and calculations. Mean values were separated and subjected to protected Fisher's least significant difference test. Data were considered different for P values < 0.05, while data with P values ≥ 0.05 but ≤ 0.10 were considered tendencies.

3.3 Results and Discussion

3.3.1 Growth Performance by Feed Phase

Main effects of post-hatch holding.

Effects of post-hatch holding time on BWG and FI were observed after the 72 hour conditioning period. Chicks that had been held for 24 or 48 hours consumed more feed (*P*<0.01) and had greater BWG (*P*<0.01) than chicks held for 0 hours (**Table 3.2**). Increased appetite due to delayed feeding most likely caused the increased feed consumption. The increased BWG may have resulted from increased feed storage in the crop, increased amount of undigested feed present in the digestive tract, or a combination of both factors. Chicks that were held for 24 hours had the lowest FI (*P*<0.01) after the conditioning period and the least BWG compared to chicks held for 0 or 48 hours. This evidence supports the hypothesis that rapid increases in BWG after post-hatch delayed feeding may not be due to compensatory growth (Bigot et al., 2003).

Effects of post-hatch holding time on ADBWG and ADFI were evident through the end of the starter phase, by which time all chicks had experienced 18 days on feed. As post-hatch holding time increased, stepwise increases (P<0.01) in ADBWG and Gain:Feed was observed. Post-hatch holding time did not affect ADFI (**Table 3.3**). These results show that chicks with longer delayed access to feed had improved feed efficiency through the starter phase.

By the end of the grower phase all chicks had received access to feed for 35 days. The chicks that experienced 48 hours of post-hatch holding had greater ADBWG (*P*<0.01) and tended to have greater ADFI (*P*=0.06) than chicks held for 0 or 24 hours (**Table 3.4**). Chicks that had been held for 24 or 48 hours had the same Gain: Feed and it was greater (P<0.01) than chicks were held for 0 hours. These results suggest that chicks delayed post-hatch access to feed maintained better feed efficiency through the grower phase.

Due to post-hatch holding time, chicks held for 0, 24 and 48 hours received 7, 5, and 6 days on the finisher phase, respectively. Chicks held for 48 hours had the greatest ADBWG (*P*<0.01) (**Table 3.5**). No effect of post-hatch holding time was observed on ADFI. Chicks held for 48 hours had a higher Gain: Feed (P<0.01) than chicks held for 0 hours, while chicks held for 24 hours had moderate Gain: Feed (**Table 3.5**). Based on these results, a long-term effect of 48 hours delayed feeding was greater BWG and better feed efficiency through market age. It has been demonstrated that male broilers from the same genetic line and fed the same diet can express different feed efficiency phenotypes due to differences in mitochondrial function (Bottje et al., 2006). Moreover, it has been suggested that delayed feeding may induce gene expression changes (Bigot et al., 2003). Based on the results of the present study, it may be possible that delayed feeding influences the feed efficiency phenotype of broilers.

Main effects of feeding strategy

At the end of the conditioning period, chicks on the PN feeding strategy had greater BWG (P<0.05) and tended to consume more feed (P=0.09) than chicks assigned the control feeding strategy (**Table 3.2**). Through the starter phase, PN chicks continued to have greater ADBWG and ADFI (P<0.01). No difference in Gain: Feed was observed (**Table 3.3**). At the end of the grower phase, feeding strategy no longer had an effect on ADBWG, although chicks on the PN feeding strategy still had higher ADFI(P<0.05) than those on the control feeding strategy (**Table 3.4**). PN chicks also had lower Gain: Feed (P<0.05) than control chicks.

Through the finisher phase, feeding strategy did not affect ADBWG (**Table 3.5**). Chicks on the PN feeding strategy had significantly greater (P<0.01) ADFI and lower (P<0.01) Gain: Feed compared to chicks on the control feeding strategy (**Table 3.5**). The PN feeding strategy utilizes diets that are formulated to have reduced nutrients. Therefore, in order to meet their nutrient requirements, the chicks needed to consume more feed, which led to the observed increase in ADFI and poorer Gain: Feed. This agrees with previous research that broilers given diets with lower nutrient density had correspondingly higher feed consumption and poorer feed conversion (Ferket et al., 2013; Saleh et al., 2004).

No interactive effects of post-hatch holding and feeding strategy were observed on performance during dietary conditioning, the starter phase, the grower phase, or the finisher phase (**Table 3.2**, **Table 3.3**, **Table 3.4**, and **Table 3.5**).

3.3.2 Growth Performance by Age

Main effects of post-hatch holding.

Through 19 days of age, chicks previously held for 48 hours post-hatch had the least BWG and FI ($P<0.01$), but the highest Gain: Feed ($P<0.01$) compared to chicks that had been held for 0 or 24 hours (**Table 3.6**), meaning that although they were not as heavy as the other chicks and they were more feed efficient. This agrees with results previously discussed and demonstrated (**Table 3.3**).

Through 36 days of age, there were no longer any effects of delayed feeding on BWG, although chicks held for 24 or 48 hours had less FI ($P<0.01$) compared to chicks that had been held for 0 hours. This resulted in a stepwise increase in Gain: Feed the longer chicks were delayed feed post-hatch (**Table 3.7**).

At the end of the study or when chicks reached 42 days of age, there were no differences in BWG and FI. However, there was a long-term effect of 48 hours post-hatch holding time on improved feed conversion ($P<0.01$) compared to chicks that had been held for 0 or 24 hours (**Table 3.8**).

Main effects of feeding strategy.

Through 19 days of age, chicks on the PN feeding strategy had greater BWG and FI (P<0.01), than chicks on the control feeding strategy (**Table 3.6**). When chicks reached 36 days of age, the PN feeding strategy no longer had an effect on BWG even though there was still greater FI (P<0.01). At this age, PN chicks began to exhibit lower Gain: Feed (P<0.01) than control chicks (**Table 3.7**). For chicks on the PN feeding strategy, these effects on FI and Gain: Feed persisted through market age or 42 days (**Table 3.8**). Reduced nutrient density in the diet formulations of the PN feeding strategy apparently led to greater feed consumption and lower feed efficiency. Diets formulated with lower nutrient density has been shown to have this affect in broilers (Ferket et al., 2013; Saleh et al., 2004).

No interactive effects of post-hatch holding and feeding strategy were observed on performance during dietary conditioning, the starter phase, the grower phase, or the finisher phase (**Table 3.6**, **Table 3.7**, and **Table 3.8**).

3.3.3 Carcass Yields

Main effects of post-hatch holding time

There were no effects of post-hatch holding time on the carcass yields of broiler chicks, although chicks that were held for 24 or 48 hours tended to have lower (*P*=0.06) wing yield than chicks that were held for 0 hours (**Table 3.9**).

Main effects of feeding strategy

Chicks on the PN feeding strategy had greater (*P*<0.05) wing yield than chicks on the control feeding strategy. In a separate study, the PN feeding strategy improved Ross 308 broilers breast yield at market age (Ferket et al., 2013). Despite the variable results of the PN feeding strategy on carcass yields, these results suggest that the PN feeding strategy may influence carcass yields.

There were no interactive effects of post-hatch holding time and feeding strategy on the carcass yields of broiler chicks, although there tended to be an interactive effect on fat pad yield.

3.3.4 Bone Quality

Although there were no effects of feeding strategy on bone breaking strength (**Table 3.10**), chicks on the PN feeding strategy had significantly lower tibia ash percentage ($P<0.05$) than chicks on the control feeding strategy (**Table 3.11**). This may be explained by reduced trace mineral concentration in the tibia ash shown in **Table 3.12**. No effect of post-hatch holding was observed on bone breaking strength or tibia ash. There were no interactive effects of post-hatch holding time and feeding strategy on the bone quality of broiler chicks at 42 days of age

3.3.5 Tissue Mineral Concentration

Main effects of post-hatch holding time

Delayed feeding of 48 hours tended ($P=0.06$) to increase the iron concentration in the tibia ash of broiler chicks. In the breast muscle, chicks held for 0 hours had higher iron concentration($P<0.05$) than chicks held for 24 hours, but it was not different from chicks held for 48 hours (**Table 3.12**, **Table 3.13**).

Main effects of feeding strategy

Chicks given the PN feeding strategy had lower manganese and zinc concentration in their tibia ash ($P<0.05$) and tended to have higher iron concentration

(P=0.07) compared to chicks on the control feeding strategy (**Table 3.12**). Despite reduction of available phosphorus and calcium in the PN diets, phosphorus and calcium concentration in the tibia ash was not affected by feeding strategy. This is most likely due to the supplemental enzymes of the PN diet. Previous research has demonstrated that certain dietary enzymes in poultry diets improve phosphorus availability (Huff et al., 1998; Scott et al., 1999)

The diets of the PN feeding strategy were formulated to supply copper, iron, manganese and zinc in an organically chelated form at 25% less than the level of the diets that were part of the control feeding strategy. Although it is known that chelated organic minerals are more bioavailable (O'Dell, 1972), the level of manganese and zinc in the PN diet may not be adequate.

Breast selenium concentration was higher (P<0.05) in chicks fed the PN feeding strategy (**Table 3.13**). This may be due to increased FI observed in chicks that consumed PN diets as part of the PN feeding strategy. There were no interactive effects of post-hatch holding time and feeding strategy on mineral concentration in tibia ash or breast muscle of broiler chicks at 42 days of age.

3.3.6 Meat Quality

Main effects of post-hatch holding time

No main effect of post-hatch holding time was observed for meat quality, although increasing post-hatch holding time tended to increase breast filet drip loss after 3 days of storage (P=0.07) (**Table 3.14**).

Main effects of feeding strategy

Feeding strategy did not have an effect on drip loss of breast filets during refrigeration of up to 7 days, but breast filets from chicks on the PN feeding strategy had higher oxidation after 7 days of refrigeration ($P<0.05$) compared to breast filets from chicks on the control feeding strategy (**Table 3.15**). Frozen thighs from PN chicks also showed greater oxidation after 5 months of freezer storage (**Table 3.16**). Even though the PN feeding strategy includes antioxidant supplements, the meat from the PN chicks was found to undergo more oxidation during storage compared to meat from the control chicks ($P<0.05$). These results were unexpected since the selenium concentration in breast meat of PN chicks was higher than that of the control chicks ($P<0.05$, **Table 3.13**). Selenium is involved in antioxidant protection against damaging reactive oxygen species (ROS) formation. Although selenium is known to spare the antioxidant requirement for vitamin E, the reduction of vitamin E in the PN diets may be too drastic for selenium to compensate. Cellular energy comes from ATP synthesis, which is a process that occurs in the mitochondria of cells. Broilers with low feed efficiency were found to have greater electron leakage and electron transport defects than broilers with higher feed effeciency during ATP synthesis (Bottje et al., 2006). The greater amount of electron leakage and defects during electron transport may have a direct effect of increasing reactive oxygen species (ROS) and is believed to lead to higher protein oxidation in tissues. Recalling the performance results of this study, PN fed chicks had significantly poorer feed conversion than control fed chicks ($P<0.01$, **Table 3.5** and **Table 3.8**). Their poorer feed efficiency caused by the PN feeding strategy may have altered mitochondrial function in the skeletal muscle of broilers enough so that the PN feeding strategy could not provide enough protection against oxidation during long storage. Furthermore, under certain circumstances, other antioxidant nutrients, such as vitamin C, are known to be antagonist to the protective effects of selenium (Watts, 1994). It is unclear if the PN diet contains nutrients or feed additives that have antagonistic properties.

No interactive effects of post-hatch holding time and feeding strategy were observed on the meat quality of broiler chicks.

3.4 Conclusions

Delayed feeding reduced early body weight gain and feed intake of broiler chicks. However, broiler chicks were able to recover by market age and those with 48 hours of delayed feed exhibited improved feed conversion than chicks that had immediate access to feed or access after 24 hours. The PN feeding strategy improved early body weight gain in broilers, yet the effect was not maintained later on in life. Broilers given the PN feeding strategy continued to consume more feed and had poorer feed conversion through market age. The PN feeding strategy was also found to influence carcass characteristics. Based on the performance, tissue mineral concentration and meat quality observations, the PN feeding strategy may alter nutrient metabolism. Further investigation on how the PN feeding strategy effects nutrient metabolism mechanisms should be considered.

3.5 Figures and Tables

Table 3.1 Composition and calculated analysis of starter, grower, and finisher diets

	Starter[1]		Grower[2]		Finisher[3]	
Diet Composition, %	Control	PN	Control	PN	Control	PN
Corn	57.00	58.84	62.42	64.08	67.12	69.07
Soybean meal (48% CP)	36.00	35.70	30.90	30.70	26.40	26.00
Vegetable oil	2.82	1.38	2.80	1.38	2.84	1.38
Limestone	1.42	1.47	1.27	1.40	1.36	1.42
Dicalcium Phosphate	1.75	1.20	1.58	1.01	1.43	0.88
Salt	0.45	0.45	0.45	0.45	0.45	0.45
L-Lysine HCL	0.10	0.11	0.09	0.09	0.00	0.00
DL-Methionine	0.21	0.20	0.24	0.24	0.15	0.15
Vitamin-mineral premix	0.25	0.00	0.25	0.00	0.25	0.00
Vitamin premix (no Vit E)	0.00	0.25	0.00	0.25	0.00	0.25
PN Premix	0.00	0.40	0.00	0.40	0.00	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME, kcal/kg	3051	2977	3108	3031	3152	3077
CP, %	22.52	22.53	20.52	20.56	18.60	18.57
Ca, %	1.03	0.93	0.93	0.85	0.92	0.82
P, avail., %	0.46	0.36	0.42	0.31	0.38	0.28
Na, %	0.29	0.29	0.28	0.28	0.26	0.26
Arg, %	1.61	1.61	1.45	1.45	1.31	1.30
Lys, %	1.36	1.36	1.20	1.20	1.00	0.99
Met+Cys, %	0.93	0.92	0.90	0.91	0.77	0.77
Thr, %	0.91	0.91	0.83	0.84	0.77	0.77
Trp, %	0.29	0.29	0.26	0.26	0.24	0.24

[1]Fed for 15 days following post-hatch holding times of 0, 24 or 48 hours.
[2]Fed for 17 days after feeding of starter diets.
[3]Fed for 7, 6, or 5 days after feeding of grower diets respective of post-hatch holding times of 0, 24, or 48 hours.

Table 3.2 Effects of post-hatch holding time and feeding strategy on body weight gain and feed intake of broiler chicks after the 72 hour conditioning period*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b
Control	34.0^{b}	39.4^{y}
PN	53.9^{a}	41.2^{x}
SEM	1.52	0.74
P-value	<0.01	0.09
Holding Main Effects		
0 h	37.6^{b}	37.5^{b}
24 h	45.6^{a}	41.3^{a}
48 h	48.8^{a}	42.2^{a}
SEM	1.84	0.90
P-value	<0.01	<0.01
Interactive Effects		
0h x Control	26.6	35.1
24h x Control	36.7	41.1
48h x Control	38.9	42.0
0h x PN	48.6	40.0
24h x PN	54.5	41.4
48h x PN	58.6	42.3
SEM	2.64	1.29
P-value	0.73	0.13

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.01$).

[xy]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

Table 3.3 Effects of post-hatch holding time and feeding strategy on the average daily BWG, FI and Gain: Feed of broiler chicks through the starter phase (18 days on feed)*

Feeding Strategy Main Effects	ADBWG, g/b/d	ADFI, g/b/d	Gain: Feed, g/g
Control	30.8[b]	44.2[b]	0.70
PN	34.6[a]	50.7[a]	0.69
SEM	0.47	0.86	0.01
P-value	<0.01	<0.01	0.48
Holding Main Effects			
0 h	30.6[c]	48.1	0.64[c]
24 h	32.7[b]	47.4	0.69[b]
48 h	34.7[a]	47.0	0.74[a]
SEM	0.57	1.06	0.01
P-value	<0.01	0.75	<0.01
Interactive Effects			
0h x Control	28.5	43.9	0.65
24h x Control	30.7	44.2	0.70
48h x Control	33.2	44.7	0.74
0h x PN	32.7	52.3	0.63
24h x PN	34.7	50.5	0.69
48h x PN	36.3	49.2	0.74
SEM	0.81	1.50	0.02
P-value	0.77	0.43	0.82

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[abc]Means within the same column without common letters are different ($P<0.05$).

Table 3.4 Effects of post-hatch holding time and feeding strategy on average daily BWG, FI and Gain: Feed of broiler chicks through the grower phase (35 days on feed)*

Feeding Strategy Main Effects	ADBWG, g/b/d	ADFI, g/b/d	Gain:Feed, g/g
Control	54.7	85.6[b]	0.64[a]
PN	55.9	91.1[a]	0.61[b]
SEM	0.56	0.90	0.00
P-value	0.16	<0.01	<0.01
Holding Main Effects			
0 h	53.2[b]	87.2[y]	0.61[b]
24 h	55.2[b]	87.3[y]	0.63[a]
48 h	57.5[a]	90.6[x]	0.64[a]
SEM	0.69	1.11	0.01
P-value	<0.01	0.06	<0.01
Interactive Effects			
0h x Control	53.2	84.5	0.63
24h x Control	54.6	84.8	0.64
48h x Control	56.5	87.5	0.65
0h x PN	53.3	89.9	0.59
24h x PN	55.9	89.8	0.62
48h x PN	58.5	93.7	0.62
SEM	0.97	1.57	0.01
P-value	0.62	0.94	0.46

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.001$).

[xy]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

Table 3.5 Effects of post-hatch holding time and feeding strategy on average daily BWG, FI and Gain: Feed through the finisher phase*

Feeding Strategy Main Effects	ADBWG, g/b/d	ADFI, g/b/d	Gain:Feed, g/g
Control	64.4	108[b]	0.59[a]
PN	64.1	113[a]	0.57[b]
SEM	0.75	1.11	0.01
P-value	0.78	<0.01	<0.01
Holding Main Effects			
0 h	62.1[b]	110	0.56[b]
24 h	63.7[b]	109	0.58[ab]
48 h	66.9[a]	112	0.60[a]
SEM	0.92	1.36	0.01
P-value	<0.01	0.33	<0.01
Interactive Effects			
0h x Control	62.7	108	0.58
24h x Control	63.4	107	0.59
48h x Control	67.1	109	0.62
0h x PN	61.6	112	0.55
24h x PN	64.0	111	0.57
48h x PN	66.7	115	0.58
SEM	1.30	1.93	0.01
P-value	0.81	0.79	0.54

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.01$).

Table 3.6 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-19 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain:Feed, g/g
Control	492[b]	712[b]	0.70
PN	556[a]	825[a]	0.68
SEM	8.02	15.3	0.01
P-value	<0.01	<0.01	0.46
Holding Main Effects			
0 h	551[a]	866[a]	0.64[c]
24 h	525[ab]	763[b]	0.69[b]
48 h	498[b]	677[c]	0.74[a]
SEM	9.83	18.7	0.02
P-value	<0.01	<0.01	<0.01
Interactive Effects			
0h x Control	514	790	0.65
24h x Control	493	706	0.70
48h x Control	470	639	0.74
0h x PN	588	942	0.63
24h x PN	556	819	0.68
48h x PN	526	714	0.74
SEM	13.9	26.5	0.02
P-value	0.80	0.36	0.76

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[abc]Means within the same column without common letters are different ($P<0.05$).

Table 3.7 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-36 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain:Feed, g/g
Control	1833	2848[b]	0.644[a]
PN	1873	3028[a]	0.620[b]
SEM	19.30	28.79	0.004
P-value	0.152	<0.001	<0.001
Holding Main Effects			
0 h	1864	3052[a]	0.611[c]
24 h	1850	2922[b]	0.634[b]
48 h	1847	2840[b]	0.650[a]
SEM	23.64	35.26	0.005
P-value	0.866	0.001	<0.001
Interactive Effects			
0h x Control	1862	2957	0.630
24h x Control	1826	2834	0.643
48h x Control	1813	2754	0.659
0h x PN	1866	3146	0.593
24h x PN	1874	3010	0.625
48h x PN	1881	2927	0.641
SEM	33.43	49.86	0.007
P-value	0.617	0.983	0.304

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[abc]Means within the same column without common letters are different ($P<0.05$).

Table 3.8 Effects of post-hatch holding time and feeding strategy on growth performance of broiler chicks through 1-42 days of age*

Feeding Strategy Main Effects	BWG, g/b	FI, g/b	Gain:Feed, g/g
Control	2638	4437[b]	0.595[a]
PN	2626	4621[a]	0.568[b]
SEM	30.79	45.82	0.006
P-value	0.781	0.008	0.002
Holding Main Effects			
0 h	2609	4615	0.564[b]
24 h	2610	4486	0.581[ab]
48 h	2677	4486	0.598[a]
SEM	37.71	56.11	0.007
P-value	0.357	0.187	0.005
Interactive Effects			
0h x Control	2632	4544	0.579
24h x Control	2598	4401	0.589
48h x Control	2684	4367	0.616
0h x PN	2586	4686	0.550
24h x PN	2622	4571	0.574
48h x PN	2669	4606	0.580
SEM	53.32	79.36	0.010
P-value	0.801	0.822	0.542

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.05$).

Table 3.9 Effects of post-hatch holding time and feeding strategy on carcass yields of broiler chicks*

Feeding Strategy Main Effects	Hot Carcass[1]	Cold Carcass[1]	Fat Pad[1]	Breast Filet[2]	Breast Tender[2]	Wing[2]	Leg Quarter[2]
Control	71.8	72.6	1.03	25.5	5.06	10.2^{b}	40.2
PN	71.7	72.9	0.97	25.9	5.15	10.4^{a}	39.3
SEM	0.26	0.18	0.04	0.27	0.06	0.07	0.46
P-value	0.82	0.32	0.24	0.33	0.28	0.04	0.16
Holding Main Effects							
0 h	71.8	73.0	1.01	25.3	5.13	10.5^{x}	39.5
24 h	71.9	72.7	1.05	25.6	5.05	10.2^{y}	40.2
48 h	71.5	72.6	0.94	26.2	5.14	10.2^{y}	39.5
SEM	0.32	0.22	0.43	0.33	0.07	0.08	0.56
P-value	0.77	0.47	0.19	0.12	0.60	0.06	0.62
Interactive Effects							
0h x Control	71.6	72.7	1.05^{x}	24.9	5.05	10.3	40.5
24h x Control	72.3	72.6	1.00^{xy}	25.5	5.13	10.2	40.3
48h x Control	71.4	72.5	1.04^{x}	26.1	5.01	10.1	39.8
0h x PN	71.9	73.3	0.97^{xy}	25.6	5.21	10.6	38.4
24h x PN	71.4	72.7	1.10^{x}	25.7	4.98	10.3	40.0
48h x PN	71.6	72.7	0.84^{y}	26.3	5.26	10.3	39.3
SEM	0.46	0.32	0.06	0.46	0.10	0.12	0.80
P-value	0.36	0.68	0.05	0.82	0.12	0.90	0.50

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.05$).

[xy]Means within the same column without common letter tend to differ ($0.05 \leq P \leq 0.10$).

[1]Values expressed as a percentage of live weight.

[2]Values expressed as a percentage of cold carcass weight

Table 3.10 Effects of post-hatch holding time and feeding strategy on bone breaking strength of broiler chicks at 42 days of age*, kg force

Feeding Strategy Main Effects	Tibia	Humerus
Control	52.39	47.89
PN	49.33	44.77
SEM	1.430	1.527
P-value	0.140	0.158
Holding Main Effects		
0 h	50.00	47.29
24 h	50.12	46.98
48 h	52.46	44.72
SEM	1.752	1.870
P-value	0.538	0.575
Interactive Effects		
0h x Control	50.67	48.19
24h x Control	50.75	47.51
48h x Control	55.75	47.97
0h x PN	49.33	46.40
24h x PN	49.49	46.45
48h x PN	49.18	41.47
SEM	2.477	2.644
P-value	0.478	0.543

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

Table 3.11 Effect of post-hatch holding time and feeding strategy on tibia ash percent of broiler chicks at 42 days of age[*]

Feeding Strategy Main Effects	Tibia Ash, %
Control	54.44[a]
PN	53.72[b]
SEM	0.174
P-value	0.006
Holding Main Effects	
0 h	54.21
24 h	53.81
48 h	54.22
SEM	0.213
P-value	0.316
Interactive Effects	
0h x Control	54.56
24h x Control	54.28
48h x Control	54.49
0h x PN	53.87
24h x PN	53.35
48h x PN	53.94
SEM	0.297
P-value	0.820

[*]Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.05$).

Table 3.12 Effects of post-hatch holding time and feeding strategy on mineral concentration of broiler chick tibia ash at 42 days of age*

Feeding Strategy Main Effects	Cu, mg/kg	Fe, mg/kg	Mn, mg/kg	Zn, mg/kg	P, %	Ca, %
Control	2.72	356^{y}	6.26^{a}	331^{a}	18.9	38.3
PN	2.78	385^{x}	5.04^{b}	295^{b}	18.9	38.2
SEM	0.04	11.0	0.15	2.83	0.04	0.09
P-value	0.27	0.07	<0.01	<0.01	0.94	0.77
Holding Main Effects						
0 h	2.76	352^{y}	5.74	308	18.9	38.2
24 h	2.71	363^{xy}	5.47	312	18.8	38.2
48 h	2.78	397^{x}	5.74	317	18.9	38.3
SEM	0.05	13.5	0.18	3.47	0.05	0.11
P-value	0.50	0.06	0.48	0.21	0.68	0.79
Interactive Effects						
0h x Control	2.78	329	6.59	323	18.9	38.2
24h x Control	2.60	334	5.96	333	18.9	38.4
48h x Control	2.78	405	6.24	338	18.9	38.2
0h x PN	2.75	374	4.90	294	18.9	38.3
24h x PN	2.81	392	4.98	292	18.8	38.0
48h x PN	2.78	389	5.24	297	19.0	38.4
SEM	0.07	19.1	0.25	4.91	0.07	0.16

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).
[ab]Means within the same column without common letters are different ($P<0.05$).
[xy]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

Table 3.13Effects of post-hatch holding time and feeding strategy on breast mineral concentration of broiler chicks at 42 days of age*

Feeding Strategy Main Effects	Cu, µg/kg	Fe, mg/kg	Mn, µg/kg	Zn, mg/kg	Se, µg/kg
Control	208	4.82	119	9.07	192^{b}
PN	217	4.63	149	8.83	254^{a}
SEM	17.9	0.17	27.2	0.35	4.94
P-value	0.72	0.45	0.44	0.63	<0.01
Holding Main Effects					
0 h	224	5.15^{a}	175	8.54	232
24 h	206	4.27^{b}	111	8.84	219
48 h	209	4.75^{ab}	117	9.48	217
SEM	21.8	0.21	32.9	0.43	6.05
P-value	0.84	0.02	0.34	0.29	0.17
Interactive Effects					
0h x Control	201	5.21	117	8.73	203
24h x Control	230	4.40	116	9.14	189
48h x Control	193	4.85	125	9.35	183
0h x PN	246	5.10	233	8.36	262
24h x PN	182	4.14	106	8.54	249
48h x PN	224	4.65	109	9.60	251
SEM	31.1	0.30	47.1	0.60	8.56
P-value	0.30	0.97	0.31	0.77	0.83

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[ab]Means within the same column without common letters are different ($P<0.05$).

Table 3.14 Effects of post-hatch holding time and feeding strategy on drip loss of raw breast filets*

Feeding Strategy Main Effects	3d drip loss, %[1]	7d drip loss, %[1]
Control	1.31	2.53
PN	1.14	2.17
SEM	0.10	0.18
P-value	0.22	0.17
Holding Main Effects		
0 h	1.07[y]	2.10
24 h	1.14[xy]	2.24
48 h	1.45[x]	2.71
SEM	0.12	0.23
P-value	0.07	0.15
Interactive Effects		
0h x Control	1.25	2.43
24h x Control	1.04	2.12
48h x Control	1.64	3.04
0h x PN	0.90	1.76
24h x PN	1.25	2.36
48h x PN	1.26	2.38
SEM	0.17	0.32
P-value	0.17	0.28

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[1]Expressed as a percentage of original breast filet weight.

[xy]Means within the same column without common letters tend to differ ($0.05 \leq P \leq 0.10$).

Table 3.15 Effects of post-hatch holding time and feeding strategy on the oxidative stability of raw breast meat during refrigerated storage[*]

Feeding Strategy Main Effects	0d TBARS[1]	3d TBARS[1]	7d TBARS[1]
Control	0.126	0.171	0.305[b]
PN	0.135	0.201	0.369[a]
SEM	0.010	0.013	0.016
P-value	0.494	0.111	0.007
Holding Main Effects			
0 h	0.127	0.182	0.346
24 h	0.136	0.197	0.350
48 h	0.129	0.178	0.315
SEM	0.012	0.016	0.020
P-value	0.862	0.656	0.159
Interactive Effects			
0h x Control	0.110	0.157	0.282
24h x Control	0.141	0.180	0.335
48h x Control	0.127	0.176	0.297
0h x PN	0.144	0.208	0.410
24h x PN	0.131	0.215	0.366
48h x PN	0.131	0.179	0.333
SEM	0.017	0.022	0.028
P-value	0.420	0.560	0.159

[*]Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).
[1]Expressed as mg malondialdehyde/kg meat.
[ab]Means within the same column without common letters are different (P<0.05).

Table 3.16 Effects of post-hatch holding time and feeding strategy on the oxidative stability of thigh meat after 5 months of frozen storage*

Feeding Strategy Main Effects	0d TBARS[1]	3d TBARS[1]	7d TBARS[1]
Control	0.245[b]	0.560	1.440
PN	0.383[a]	0.777	1.400
SEM	0.038	0.118	0.217
P-value	0.022	0.201	0.896
Holding Main Effects			
0 h	0.253	0.637	1.678
24 h	0.292	0.625	1.208
48 h	0.396	0.743	1.375
SEM	0.047	0.144	0.266
P-value	0.116	0.816	0.465
Interactive Effects			
0h x Control	0.166	0.552	1.610
24h x Control	0.217	0.435	1.114
48h x Control	0.351	0.695	1.597
0h x PN	0.341	0.723	1.746
24h x PN	0.367	0.815	1.301
48h x PN	0.441	0.792	1.152
SEM	0.066	0.204	0.375
P-value	0.811	0.773	0.654

*Mean values represent pen averages (Feeding Strategy main effects, n=24; Holding main effects, n=16; Interactive effects, n=8).

[1]Expressed as mg malondialdehyde/kg meat.

[ab]Means within the same column without common letters are different ($P<0.05$).

CHAPTER 4 – Effect of breed/strain and feeding strategy on early growth performance, bone strength, and immune organ weight of young meat-type chickens

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4.1 Abstract

Very little information exists on the nutrient requirements, growth, and development of slow-growing heritage breeds or moderate to fast growing poultry strains used for pasture poultry meat production. The Programmed Nutrition (PN) feeding strategy utilizes a 72 hour proprietary conditioning diet designed to allow chicks to adapt to reduced nutrient diets later on in life. The present study was designed to determine the effects of breed/strain and early feeding strategy on growth performance, bone quality and relative bursal weight of young meat-type chickens. The five meat-type breeds/strains included the slow-growing, heritage breeds which were the Black Australorp, Barred Plymouth Rock, and Rhode Island Red. The two meat-type chicken strains were a moderate-growing Red Ranger strain and a fast-growing Cornish Cross strain. In a 5x2 factorial arrangement the five breeds/strains were randomly assigned to either a control feeding strategy or PN feeding strategy. The Cornish Cross strain exhibited the greatest growth performance out of all the meat-type chickens (P<0.01), with the Red Ranger strain having the second best growth performance (P<0.01) and the heritage breeds/strains having the lowest growth performance (P<0.01). Bone strength of the five breeds/strains was also complimentary to growth performance. Chicks on the PN feeding strategy had significantly reduced early growth performance and bone strength (P<0.01). However, early feeding strategy did not affect bursa organ weight. Interactive effects of breed/strain and early feeding strategy were observed for early growth performance and

bone strength, especially for the Red Ranger and Cornish Cross strains. The slower-growing, heritage breeds were less affected by early feeding strategy. The results of this experiment indicate that the PN feeding strategy may not be suitable for moderate-growing or fast-growing strains of meat-type chickens.

Key words: Programmed Nutrition feeding strategy, chicken breed/strain, performance, bone strength, immunity

Introduction

In the United States, there is a growing interest in small, backyard chicken flocks with a focus on organic and free-range meat and egg production (Fanatico et al., 2005). Evidence of this growing interest is supported by increased publications regarding backyard or small flock poultry topics as well as increased business of small hatcheries (McCrea et al., 2014). Some of these small hatcheries specialize in producing heritage chicken breeds for egg, meat, or dual purpose production. However, heritage breed chickens have a much slower growth rate than modern, commercial broiler strains which have been genetically selected for fast growth (Havenstein et al., 2003; McCrea et al., 2014; Schmidt et al., 2009; Zuidhof et al., 2014). In the mid 1900's, heritage breeds such as the Barred Plymouth Rock and Rhode Island Red, although desired for their beautiful plumage, were found to be uneconomical choices for large-scale, commercial poultry production (Warren, 1958). Despite their slower growth rate, heritage breeds such as Barred Plymouth Rock, Rhode Island Red, and Black Australorp are being utilized for pasture poultry production (Hilimire, 2012). Although some heritage breeds have been selected for egg production, there is a lack of strains developed for meat production of these breeds (Fanatico, 2008). The Cornish Cross strains are known as the fastest-growing meat-type chickens and are used in both pasture and commercial meat production. The Red Ranger strain is a medium-growing chicken that is being produced in free-range settings for local markets in southeastern United States (Smith, 2012). Due to lack of genetic selection for meat production, the nutrient requirements of slow-growing, heritage breeds and moderate-growing broiler strains are unknown. Furthermore, there is a lack of published information on the growth performance of some these breeds/strains in a modern, commercialized setting. Therefore, this objective of this study is to investigate the effects of breed/strain and early feeding strategy on the early growth performance, bone strength, and immune organ weight of young meat-type chickens.

4.2 Materials and Methods

The following study was conducted under the protocols created and approved by the University of Kentucky Institutional Animal Care and Use Committee.

4.2.1 Animals and Treatments

A total of 1800, one-day old male broiler chicks of five different meat-type chicken breeds/strains were ordered from Murray McMurray Hatchery (Webster City Hatchery) and shipped via USPS air mail. The five meat-type chickens were Barred Plymouth Rock, Black Australorp, Rhode Island Red, Red Ranger, and Cornish Cross. Upon arrival to the experimental facility, 360 chicks from each type were randomly assigned to 10 experimental treatments utilizing a 5x2 factorial arrangement based on the five breeds/strains and two feeding strategies.

Chicks were assigned to cages (6 replicate cages with 6 chicks per cage) where they were provided *ad libitum* access to water (via 2 nipple drinkers) and feed. The cage dimensions were 50.8 x 60.96 square centimeters (20 x 24 square inches). The two feeding strategies involved providing a corn-soybean meal starter (control) diet or Programmed Nutrition (PN) feeding strategy. The PN feeding strategy consisted of feeding a proprietary PN Post-Hatch Broiler (Alltech, Inc., Nicholasville, KY) conditioning diet for 72 hours followed by placement on a PN starter diet. The control diet was formulated to meet or exceed chick nutrient and energy requirements as determined by the Poultry NRC (1994), whereas the PN starter diet was formulated to contain antioxidants, enzymes and organic trace minerals, yet be lower in energy and nutrient content (**Table 4.1**).

4.2.2 Growth Performance Measurements

Chicks were weighed by cage at placement, after 72 hours, and weekly until 4 weeks of age to calculate average body weight gain (BWG). Average feed intake (FI) for each pen was also measured after 72 hours and weekly until 4 weeks of age. Feed conversion was calculated using average BWG to average FI ratio, which was expressed as Gain: Feed.

4.2.3 Bone Quality Measurements

At 28 days of age, 2 chicks from each cage were randomly selected and euthanized for collection of tibias and humeri. Bone breaking strength was measured using an Instron Testing Instrument (Model 4301). The bones were manually cleaned of excess soft tissue and placed flat on a raised platform. Approximately 100 kg force at a speed of 50 mm/sec was applied with a stainless steel wedge probe positioned perpendicular to the center of the bone shafts until they fractured.

4.2.4 Bursa Collection

When chicks reached 28 days of age, 2 chicks from each cage were randomly selected and euthanized for collection of Bursa of Fabricius (bursa). Live chick weight was recorded prior to sample collection. Bursas were removed and weighed for determination of the relative bursal weight (expressed as a percentage of body weight). Values from each cage were averaged before statistical analysis.

4.2.5 Statistical Analysis

Statistical Analysis of the data was carried out using the general linear model procedures of SAS (v9.3). Analysis of variance of the data was used to determine the main effects of breed/strain, main effects of feeding strategy, and interactive effects of breed/strain and feeding strategy on early growth performance and bone quality. Means were separated and subjected to protected Fisher's least significant difference test. Means with P values < 0.05 were considered different whereas means with P values ≥ 0.05 but ≤ 0.10 only tended to differ.

4.3 Results and Discussion

4.3.1 Growth Performance

Main effect of breed/strain on body weight gain

At placement, there was a significant difference in chick placement weight (P<0.01). Placement weights ranged from approximately 30-40 grams with the Red Ranger chicks weighing the most (39.61 g). The Cornish Cross chicks had the second greatest BW (34.64 g) at placement followed by Rhode Island Red and Black Australorp chicks (31.32 g and 31.46 g respectively). The Barred Plymouth Rock chicks weighed the least (29.66g) at placement.

After 3 days, the Cornish Cross and Red Ranger chicks had greater BWG (P<0.01) than the Rhode Island Red, Black Australorp and Bared Plymouth Rock chicks

(**Table 4.2**). However, it should be noted that the Rhode Island Red chicks also had greater BWG (P<0.01) than the Black Australorp and Barred Plymouth Rock chicks.

By day 7, the Cornish Cross chicks had the greatest BWG (P<0.01), followed by the Red Ranger and then Rhode Island Red chicks (**Table 4.2**). The Black Australorp and Barred Plymouth Rock chicks had the least BWG (P<0.01). As observed on day 7, breed/strain had the same effect on BWG of chicks at days 14, 21, and 28 (**Table 4.2**). At the end of the study (28 days), the Cornish Cross chicks weighed nearly 1.5 times more than the Red Ranger chicks and nearly 3.5 times more than the Rhode Island Red, Black Australorp, and Barred Plymouth Rock chicks. These results agree with the literature, since it has been established that the Cornish Cross strain is the fastest growing, while Red Ranger strains have moderate growth and the heritage breeds exhibit the slowest growth (Fanatico et al., 2005; Hilimire, 2012; Schmidt et al., 2009; Warren, 1958; Zuidhof et al., 2014).

Main effect of breed/strain on feed intake

After 3 days, the Cornish Cross and Red Ranger chicks had greater FI (P<0.01) than the Rhode Island Red, Black Australorp and Bared Plymouth Rock chicks (**Table 4.3**). By day 7, the Cornish Cross chicks had surpassed the Red Ranger chicks in feed consumption (P<0.01), whereas the Rhode Island Red, Black Australorp and Barred Plymouth Rock chicks continued to have the lowest FI of the five meat-type chickens (**Table 4.3**). However, at days 14 and 21, the Rhode Island Red chicks had significantly greater FI (P<0.01) than the Black Australorp and Barred Plymouth Rock chicks (**Table 4.3**). By 28 days, the Cornish Cross chicks had consumed about 1.25 times more feed than the Red Ranger chicks and about 2.5 times more feed than the Rhode Island Red, Black Australorp, and Barred Plymouth Rock chicks (**Table 4.3**).

Main effect of breed/strain on gain: feed

After 3 days, the Cornish Cross and Red Ranger chicks had greater Gain: Feed (*P*<0.01) than the Rhode Island Red, Black Australorp and Bared Plymouth Rock chicks (**Table 4.4**). However, it should be noted that the Rhode Island Red chicks also had a greater Gain: Feed (*P*<0.01) than the Black Australorp and Barred Plymouth Rock chicks. Beginning at day 7 and through day 21, the Cornish Cross chicks had the greatest Gain: Feed (*P*<0.01), followed by the Red Ranger chicks and then the Rhode Island Red chicks. The Black Australorp and Barred Plymouth Rock chicks had the poorest feed conversion (*P*<0.01, **Table 4.4**). By day 28, the Rhode Island Red chicks had greater Gain: Feed (*P*<0.01) compared to the Barred Plymouth Rock chicks, although the feed conversion of both breeds did not differ from that of the Black Australorp breed.

Main effect of feeding strategy on growth performance

Feeding strategy did not have an effect on BWG of chicks until 14 days of age. From 14 until 28 days of age, chicks assigned to the control feeding strategy were heavier (*P*<0.05) than chicks assigned to the PN feeding strategy (**Table 4.2**). At 7 days of age, chicks on the PN feeding strategy had greater FI (*P*<0.05) than chicks fed the control feeding strategy (**Table 4.3**). At day 7 of age, chicks on the PN feeding strategy experienced a decline in Gain: Feed (*P*<0.05), whereas chicks on the control feeding strategy began to exhibit greater Gain: Feed (*P*<0.05) (**Table 4.4**). After day 7, control chicks maintained a greater feed conversion (*P*<0.01) than PN chicks. These results indicate that PN chicks were less efficient in utilizing the feed for growth which may be a consequence of the reduced energy and nutrients in the PN starter diet.

Interactive effects of breed/strain and feeding strategy on body weight gain

Interactive effects of breed/strain and feeding strategy on BWG of chicks was observed at days 14, 21 and 28 for Cornish Cross and Red Ranger strains. At 14 days of age, the Cornish Cross chicks had the greatest BWG (P<0.01) of all the meat-type chicks regardless of feeding strategy, although the PN feeding strategy was found to reduce BWG (**Table 4.2**). The Red Ranger chicks given the control feeding strategy had greater BWG (P<0.01) than the Red Ranger chicks assigned to the PN feeding strategy. At days 21 and 28, the Cornish Cross chicks on the control feeding strategy were heavier (P<0.01) than the Cornish Cross chicks given the PN feeding strategy. The Red Ranger chicks on the control feeding strategy remained heavier (P<0.01) than their PN counterparts. The PN feeding strategy did not have such a pronounced effect on the BWG of Rhode Island Red, Black Australorp, and Barred Plymouth Rock breeds. These results indicate that early growth performance is dependent upon breed/strain and feeding strategy.

Interactive effects of breed/strain and feeding strategy on feed intake

Interactive effects of breed/strain and feeding strategy on FI were only observed on day 21. Cornish Cross chicks consumed the most feed regardless of feeding strategy provided (**Table 4.3**). The Red Ranger chicks on the control feeding strategy consumed more feed (P<0.05) than the Red Ranger chicks on the PN feeding strategy, whereas the PN Barred Plymouth Rock chicks consumed more feed (P<0.05) than the control Barred

Plymouth Rock chicks. The breeds and strains that were found to consume more also had greater BWG (**Table 4.2** and **Table 4.3**).

Interactive effects of breed/strain and feeding strategy on feed conversion

There were no significant interactive effects of breed/strain and feeding strategy on feed conversion although there tended to be differences on days 14 and 28 (**Table 4.4**). Cornish Cross, Red Ranger, and Black Australorp chicks provided the control feeding strategy tended to have improved feed conversion (P=0.08) than the same meat-type chicks on the PN feeding strategy on day 14, while only the Red Ranger chicks fed the control feeding strategy tended to have improved feed conversion on day 28(P=0.10) (**Table 4.4**).

4.3.2 Bone Quality

Main effect of breed/strain on bone breaking strength

At 28 days of age, the Cornish Cross chicks had the greatest (P<0.01) humerus and tibia breaking strength of all the meat-type chickens (**Table 4.5**). The Red Ranger chicks had the second greatest (P<0.01) humerus and tibia breaking strength. The Rhode Island Red, Black Australorp and Barred Plymouth Rock chicks all had similar humerus breaking strengths (**Table 4.5**). Rhode Island Red tibia breaking strength did not differ from that of the Black Australorp and Barred Plymouth Rock chicks, although Black Australorp chicks had greater tibia breaking strength than the Barred Plymouth Rock chicks (P<0.01) (**Table 4.5**). The Rhode Island Red, Black Australorp and Barred Plymouth Rock breeds had the least growth performance (**Table 4.2**, **Table 4.3**, and **Table 4.4**), smaller body size, and smaller bone size compared to the Cornish Cross and Red Ranger strains. Therefore, their poorer growth performance and body size may be related to skeletal development and may have influence the bone breaking strength results.

Main effect of feeding strategy on bone breaking strength

There was an effect of feeding strategy on both humerus and tibia breaking strength. Chicks assigned to the control feeding strategy had greater bone breaking strength (P<0.01) compared to chicks assigned to the PN feeding strategy (**Table 4.5**). This may be due to the reduced nutrient concentration, specifically calcium and phosphorus, of the PN starter diet.

Interactive effects of breed/strain and feeding strategy on bone breaking strength

Interactive effects of breed/strain and feeding strategy were observed for humerus and tibia breaking strength (**Table 4.5**). Cornish Cross and Red Ranger chicks on the control feeding strategy had greater humerus and tibia breaking strength (P<0.01) compared to the same chicks on the PN feeding strategy. Additionally, Red Ranger chicks given the control strategy had tibia breaking strength comparable to that of Cornish Cross chicks on the PN feeding strategy. Black Australorp chicks fed on the control feeding strategy had greater tibia breaking strength (P<0.01) compared to Rhode Island Red chicks on the PN feeding strategy and the Black Australorp chicks regardless of feeding strategy. Rhode Island Red, Black Australorp, and Barred Plymouth Rock chicks had the least humerus breaking strength among the five meat-type chickens regardless of feeding strategy (P<0.01, **Table 4.5**). Depending on the breed/strain, the PN feeding strategy was shown to negatively influence bone breaking strength for moderate-growing and fast-growing strains. Feeding strategy did not have such an effect on the slower-growing, heritage breeds.

Due to the different growth performance and body size of the five meat-type chickens during the experiment, it very likely that bone strength is influence by breed/strain as well as feeding strategy.

4.3.3 Relative Bursal Weight

Main effect of breed/strain on relative bursal weight

Rhode Island Red chicks had greater relative bursal weights ($P<0.01$) than the other meat-type chicks except for the Black Australorp, whereas the Black Australorp chicks only had a greater relative bursal weight ($P<0.01$) than the Cornish Cross chicks (**Table 4.6**). These results indicate that Rhode Island Red chicks may have a greater immune capacity compared to other meat-type chickens.

There were no effects of feeding strategy on relative bursal weight. .No interactive effects of breed/strain and feeding strategy on relative bursal weight were observed during the study.

4.4 Conclusions

Based on the performance results by breed/strain, the Cornish Cross strain was found to have superior growth and feed conversion compared to the other four meat-type chickens. The Cornish Cross strain is also the most similar to modern, commercial broiler strains. The Red Ranger strain had the second greatest growth and feed conversion, whereas heritage breeds (Barred Plymouth Rock, Black Australorp and Rhode Island Red breeds) had the least growth and feed conversion. The slowest growing breeds were also had the weakest bone strength. The Rhode Island Red breed had the heaviest bursal weight, which may be an indicator of higher immune capacity.

The Programmed Nutrition feeding strategy was found to have a negative effect on growth performance and bone breaking strength, but did not affect relative bursal weight. Based on the interactive effects of breed/strain and feeding strategy as well as main effects of feeding strategy on performance and bone strength, Programmed Nutrition did not have as much of an effect on the slow-growing heritage breeds compared to the moderate-growing or fast-growing meat-type strains. Therefore, Programmed Nutrition may not be a suitable early feeding strategy for faster-growing strains and it is unclear what the long-term effects are for slower-growing breeds.

4.5 Acknowledgements

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4.6 Figures and Tables

Table 4.1 Composition and calculated analysis of starter diets

	Starter Diet	
Diet Composition, %	Control	PN
Corn	56.37	59.00
Soybean meal (48% CP)	36.50	36.70
Vegetable oil	2.70	0.80
Limestone	1.42	1.42
Dicalcium Phosphate	1.75	0.66
Salt	0.45	0.46
L-Lysine HCL	0.10	0.10
DL-Methionine	0.21	0.21
Mineral premix	0.25	0.00
Vitamin premix (no Vit E)	0.25	0.25
Vitamin E	0.03	0.00
PN Premix	0.00	0.40
Total	100.03	100.00
Calculated Analysis		
ME, kcal/kg	3031.82	2954.76
CP, %	22.71	23.03
Ca, %	1.03	0.80
P, avail., %	0.46	0.26
Na, %	0.29	0.30
Arg, %	1.63	1.65
Lys, %	1.37	1.38
Met+Cys, %	0.93	0.95
Thr, %	0.92	0.93
Trp, %	0.30	0.30

Table 4.2 Effects of breed/strain and feeding strategy on cumulative BWG (g/b) of young chickens*

Feeding Strategy Main Effects		3 d	7 d	14 d	21 d	28 d
Control		20.8^{y}	71.2	216^{a}	401^{a}	609^{a}
PN		22.3^{x}	71.2	199^{b}	373^{b}	559^{b}
SEM		0.54	1.48	3.36	5.70	7.73
P-value		0.06	0.99	<0.01	<0.01	<0.01
Breed/Strain Main Effects						
Barred Plymouth Rock		14.3^{d}	40.3^{d}	107^{d}	201^{d}	296^{d}
Black Australorp		15.0^{cd}	42.5^{d}	115^{d}	195^{d}	292^{d}
Rhode Island Red		17.5^{c}	50.0^{c}	130^{c}	231^{c}	336^{c}
Cornish Cross		32.1^{a}	129^{a}	411^{a}	803^{a}	1220^{a}
Red Ranger		28.8^{b}	94.5^{b}	276^{b}	503^{b}	777^{b}
SEM		0.86	2.34	5.31	8.98	12.2
P-value		<0.01	<0.01	<0.01	<0.01	<0.01
Interactive Effects						
Breed/Strain	Strategy					
Barred Plymouth Rock	Control	14.3	38.2	104^{g}	200^{fg}	294^{f}
Black Australorp	Control	14.2	44.4	118^{efg}	189^{g}	288^{f}
Rhode Island Red	Control	17.7	50.8	134^{e}	239^{e}	344^{e}
Cornish Cross	Control	30.2	126	422^{a}	829^{a}	1266^{a}
Red Ranger	Control	27.8	96.9	303^{c}	548^{c}	854^{c}
Barred Plymouth Rock	PN	14.3	42.3	111^{fg}	203^{efg}	297^{ef}
Black Australorp	PN	15.8	40.7	111^{fg}	202^{fg}	296^{f}
Rhode Island Red	PN	17.4	49.3	126^{ef}	224^{ef}	329^{ef}
Cornish Cross	PN	34.0	132	400^{b}	777^{b}	1174^{b}
Red Ranger	PN	29.9	92.1	249^{d}	458^{d}	699^{d}
SEM		1.21	3.31	7.50	12.7	17.2
P-value		0.40	0.39	<0.01	<0.01	<0.01

*Mean values represent cage averages (Feeding Strategy main effects, n=30; Breed/Strain main effects, n=12; Interactive effects, n=6).

$^{a\text{-}g}$Mean values within a column without common letters are different (P<0.01).

^{xy}Mean values within a column without common letters tend to be different ($0.05 \leq P \leq 0.10$).

Table 4.3 Effects of breed/strain and feeding strategy on cumulative FI (g/b) of young chickens[*]

Feeding Strategy Main Effects		3 d	7 d	14 d	21 d	28 d
Control		33.5	107^{b}	323	602	1005
PN		35.1	113^{a}	328	600	994
SEM		0.69	2.02	5.31	9.45	12.1
P-value		0.11	<0.05	0.48	0.91	0.53
Breed/Strain Main Effects						
Barred Plymouth Rock		28.7^{b}	79.2^{c}	218^{d}	351^{d}	653^{d}
Black Australorp		29.9^{b}	82.2^{c}	219^{d}	380^{d}	627^{cd}
Rhode Island Red		30.3^{b}	86.9^{c}	247^{c}	425^{c}	697^{c}
Cornish Cross		42.4^{a}	171^{a}	529^{a}	1078^{a}	1698^{a}
Red Ranger		40.1^{a}	132^{b}	413^{b}	773^{b}	1322^{b}
SEM		1.09	3.18	8.37	14.9	19.0
P-value		<0.01	<0.01	<0.01	<0.01	<0.01
Interactive Effects						
Breed/Strain	Strategy					
Barred Plymouth Rock	Control	28.3	78.1	215	319^{f}	651
Black Australorp	Control	28.8	77.6	199	359^{ef}	605
Rhode Island Red	Control	31.4	86.8	248	427^{d}	701
Cornish Cross	Control	41.7	164	526	1092^{a}	1718
Red Ranger	Control	37.4	131	425	813^{b}	1348
Barred Plymouth Rock	PN	29.1	80.4	222	383^{de}	655
Black Australorp	PN	31.1	86.9	239	401^{de}	649
Rhode Island Red	PN	29.3	86.9	246	422^{d}	693
Cornish Cross	PN	43.2	178	531	1063^{a}	1677
Red Ranger	PN	42.8	134	402	732^{c}	1296
SEM		1.55	4.49	11.8	21.1	26.9
P-value		0.22	0.55	0.15	0.01	0.43

[*]Mean values represent cage averages (Feeding Strategy main effects, n=30; Breed/Strain main effects, n=12; Interactive effects, n=6).
[a-f]Mean values within a column without common letters are different (P<0.05).

Table 4.4 Effects of breed/strain and feeding strategy on Gain: Feed (g/g) of young chickens*

Feeding Strategy Main Effects		3 d	7 d	14 d	21 d	28 d
Control		0.61	0.62^{a}	0.61^{a}	0.61^{a}	0.56^{a}
PN		0.62	0.59^{b}	0.57^{b}	0.58^{b}	0.52^{b}
SEM		0.02	0.01	0.01	0.01	0.01
P-value		0.59	<0.05	<0.01	<0.01	<0.01
Breed/Strain Main Effects						
Barred Plymouth Rock		0.50^{c}	0.49^{d}	0.48^{d}	0.52^{d}	0.45^{d}
Black Australorp		0.47^{c}	0.48^{d}	0.48^{d}	0.52^{d}	0.47^{cd}
Rhode Island Red		0.61^{b}	0.57^{c}	0.53^{c}	0.55^{c}	0.48^{c}
Cornish Cross		0.76^{a}	0.77^{a}	0.78^{a}	0.75^{a}	0.72^{a}
Red Ranger		0.73^{a}	0.72^{b}	0.67^{b}	0.65^{b}	0.59^{b}
SEM		0.03	0.01	0.01	0.01	0.01
P-value		<0.01	<0.01	<0.01	<0.01	<0.01
Interactive Effects						
Breed/Strain	Strategy					
Barred Plymouth Rock	Control	0.50	0.49	0.48^{z}	0.52	0.45^{xy}
Black Australorp	Control	0.44	0.49	0.50^{xyz}	0.53	0.48^{xy}
Rhode Island Red	Control	0.62	0.59	0.54^{w}	0.56	0.49^{x}
Cornish Cross	Control	0.73	0.78	0.81^{u}	0.76	0.74^{u}
Red Ranger	Control	0.75	0.74	0.71^{v}	0.68	0.63^{v}
Barred Plymouth Rock	PN	0.50	0.49	0.48^{yz}	0.51	0.45^{y}
Black Australorp	PN	0.51	0.47	0.47^{z}	0.51	0.46^{xy}
Rhode Island Red	PN	0.60	0.55	0.52^{xy}	0.53	0.48^{xy}
Cornish Cross	PN	0.79	0.75	0.75^{v}	0.73	0.70^{u}
Red Ranger	PN	0.70	0.69	0.62^{w}	0.62	0.54^{w}
SEM		0.04	0.02	0.02	0.01	0.01
P-value		0.50	0.78	0.08	0.67	0.10

*Mean values represent cage averages (Feeding Strategy main effects, n=30; Breed/Strain main effects, n=12; Interactive effects, n=6).
$^{a-d}$Mean values within a column without common letters are different (P<0.05).
$^{u-z}$Mean values within a column without common letters tend to be different (0.05≤P≤0.10).

Table 4.5 Effects of breed/strain and feeding strategy on bone breaking strength, kg force*

Feeding Strategy Main Effects		Tibia	Humerus
Control		14.7[a]	11.6[a]
PN		11.0[b]	9.69[b]
SEM		0.40	0.21
P-value		<0.01	<0.01
Breed/Strain Main Effects			
Barred Plymouth Rock		6.56[d]	5.66[c]
Black Australorp		8.32[c]	5.81[c]
Rhode Island Red		7.63[cd]	5.97[c]
Cornish Cross		24.3[a]	21.6[a]
Red Ranger		17.5[b]	14.2[b]
SEM		0.62	0.33
P-value		<0.01	<0.01
Interactive Effects			
Breed/Strain	Strategy		
Barred Plymouth Rock	Control	6.74[e]	5.67[e]
Black Australorp	Control	9.36[d]	6.30[e]
Rhode Island Red	Control	8.65[de]	6.60[e]
Cornish Cross	Control	28.7[a]	23.9[a]
Red Ranger	Control	20.0[b]	15.5[c]
Barred Plymouth Rock	PN	6.39[e]	5.65[e]
Black Australorp	PN	7.29[de]	5.31[e]
Rhode Island Red	PN	6.60[e]	5.33[e]
Cornish Cross	PN	20.0[b]	19.3[b]
Red Ranger	PN	14.9[c]	12.8[d]
SEM		0.88	0.47
P-value		<0.01	<0.01

*Mean values represent cage averages (Feeding Strategy main effects, n=30; Breed/Strain main effects, n=12; Interactive effects, n=6).

[a-e]Mean values within a column without common letters are different (*P*<0.01).

Table 4.6 Effects of breed/strain and feeding strategy on relative bursal weight*

Feeding Strategy Main Effects		Relative bursal weight[1], %
Control		0.33
PN		0.35
SEM		0.03
P-value		0.67
Breed/Strain Main Effects		
Barred Plymouth Rock		0.32[bc]
Black Australorp		0.39[ab]
Rhode Island Red		0.53[a]
Cornish Cross		0.19[c]
Red Ranger		0.28[bc]
SEM		0.05
P-value		<0.01
Interactive Effects		
Breed/Strain	Strategy	
Barred Plymouth Rock	Control	0.33
Black Australorp	Control	0.30
Rhode Island Red	Control	0.58
Cornish Cross	Control	0.18
Red Ranger	Control	0.27
Barred Plymouth Rock	PN	0.32
Black Australorp	PN	0.49
Rhode Island Red	PN	0.47
Cornish Cross	PN	0.19
Red Ranger	PN	0.29
SEM		0.07
P-value		0.27

*Mean values represent cage averages (Feeding Strategy main effects, n=30; Breed/Strain main effects, n=12; Interactive effects, n=6).

[a-c]Mean values within a column without common letters are different (*P*<0.01).

CHAPTER 5 – Summary and Conclusions

In the experiment described in Chapter 2, commercial broiler chicks were subjected to delayed feeding followed by placement on the Programmed Nutrition feeding strategy (PN) with or without 72 hours of dietary conditioning. Separately, delayed feeding and PN feeding strategy were found to have transient effects on early growth development. Delayed feeding of 48 hours exasperated early body weight loss along with yolk sac utilization and negatively impacted early growth performance, whereas PN feeding with conditioning improved early growth performance. However, broiler chicks did not reach their maximal growth performance potential at the end of the experiment which is thought to be due to the reduced calcium and phosphorus concentration in the PN starter, grower, and pre-harvest diets.

Although delayed feeding did not affect jejunum morphology at mid-life, long-term effects on carcass yields of commercial broiler chicks were also observed due to delayed feeding and PN feeding strategy. Delayed feeding of up to 48 hours decreased carcass yield of broilers through market age, while PN feeding with conditioning alleviated the negative effects of delayed feeding on carcass yield. No effects of delayed feeding or feeding strategy were observed for bone breaking quality, but there were interactive effects of delayed feeding and feeding strategy and bone mineral concentration, specifically for zinc. Previous research has suggested that delayed feeding and early nutrition strategy can induce long-term alterations in nutrient transport and metabolism.

In Chapter 3, commercial chicks were subjected to delayed feeding followed by placement on the PN feeding strategy or a conventional commercial feeding strategy.

The PN feeding strategy differed from the conventional commercial feeding strategy in that it employed a 72 hour dietary conditioning period before beginning starter, grower and pre-harvest diets formulated to have reduced energy and nutrient content. Based on the growth performance results in Chapter 2, the calcium and phosphorus nutrient concentration of the PN starter, grower, and pre-harvest diets were readjusted to achieve target growth performance potential. Similar to the experiment in Chapter 2, delayed feeding adversely affected early growth performance, whereas PN feeding strategy improved it. Broiler chicks reached target body weight at market age regardless of delayed feeding or feeding strategy. However, broiler chicks that were delayed feed for 48 hours showed improved best feed efficiency, whereas chicks on the PN feeding strategy exhibited poorer feed conversion.

Based on the ability of PN conditioning to mitigate the negative effects of delayed feeding on carcass yield in Chapter 2, the experiment in Chapter 3 sought to investigate the effects of delayed feeding and PN feeding strategy on both carcass yield and meat quality during storage. The PN feeding strategy improvements to carcass yield were not as profound as those observed in the Chapter 2. Only wing yield was improved for this experiment. Due to the high selenium concentration of the breast muscle of chicks on the PN feeding strategy, meat quality was expected to be higher during storage since dietary selenium is known participate in antioxidant protection against reactive oxygen species that generate during storage. However, breast meat drip loss was unaffected by PN feeding strategy and breast meat oxidation was higher after 7 days of refrigerated storage compared to the control feeding strategy. Higher oxidation in thigh meat that was frozen and stored for five months and then thawed was also observed. Although dietary selenium spares vitamin E, it is possible that the PN feeding strategy may contain inadequate Vitamin E levels or other components that may be antagonistic to protective antioxidant activity that may have led to the reduction in meat quality observed during storage.

The enzymes that are included in the PN feeding strategy may be the reason why broiler chicks demonstrated no difference in tibia phosphorus and calcium concentration, bone breaking strength, and bone ash despite reduced phosphorus and calcium concentration of the PN diets. Interestingly, delayed feeding was found to decrease iron concentration in the chicken breast whereas PN feeding strategy decreased tibia manganese and zinc concentration, thereby elucidating other long-term effects of delayed feeding and early nutrition strategy.

The experiment carried out in Chapter 4 was different from the previous chapters in that instead of delayed feeding, the effects of different meat-type breeds and strains of chicken and PN feeding strategy on early growth performance and development was evaluated. Because PN feeding strategy is an early nutrition strategy designed to maximize growth potential of broilers while using reduced nutrient diets, it was hypothesized that different meat-type chickens may also benefit from this strategy. However, the PN strategy was not found to be conducive to early growth performance and bone breaking strength of certain meat-type chickens. In fact, the PN feeding strategy had minimal effects on the growth and development of slow-growing, heritage breed chicks, but more severe negative effects on the growth and development of moderate-growing or faster-growing strains.

In conclusion, the negative consequences of delayed feeding on commercial broiler chick growth performance are only evident during early growth. If chicks are fed nutrient deficient diets, then the effects of delayed feeding can have lasting effects on carcass characteristics and bone mineral concentration. The dietary conditioning of the PN feeding strategy is able to alleviate the negative effects of delayed feeding when chicks are fed nutrient deficient diets and influence bone mineral concentration when paired with delayed feeding. Furthermore, the PN feeding strategy allows for the reduction of nutrient concentration in starter, grower, and finisher diets without affecting body weight gain. However, the PN feeding strategy may promote less than optimum feed conversion and meat quality of commercial broiler chicks as well as hinder the early growth and development of other meat-type chickens due to the reduction of essential dietary nutrients. Therefore, the nutrient levels of the PN feeding strategy diets may need to re-evaluated.

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Vita

Marquisha Paul was born on May 23, 1984 in Norfolk, Virginia to Mark Paul and Cheryl Anita Paul. Marquisha lived in Chesapeake, Virginia for most of her childhood and that is where she attended middle school and high school. She graduated from Hickory High School in 2002.

In the fall of 2002 Marquisha relocated to Frankfort, Kentucky to attend Kentucky State University. During her freshman and sophomore year Marquisha Paul was active in the Whitney Young School Honors Program and was selected to participate in several model UN conferences and leadership workshops. In her junior and senior year, she was active in Kentucky State University's National Science Foundation TEAMS program and worked as an undergraduate student research assistant to Dr. Changzheng Wang. In Dr. Wang's lab she assisted with nutrition research and was given the opportunity to conduct research on the improving the calcium content of bone soup for use as an alternative source of dietary calcium. Marquisha graduated in 2006 from Kentucky State University with an associate's degree in Liberal Studies and a bachelor's degree in Biology.

After giving birth to her first child in 2006, Marquisha decided to enter the workforce. She joined Alltech Inc., in January 2007 as a research laboratory technician where she participated in animal nutrition research. While continuing to work, Marquisha made the choice to pursue higher education. In 2012, she enrolled into the Animal and Food Science Master's program at the University of Kentucky, Lexington, Kentucky. Under the supervision of Dr. Anthony Pescatore she has conducted research in poultry nutrition. While an M.S. student Marquisha became engaged to Gustavo Cruz Hermenegildo and was married in 2015.